

National vandalism at the US Geological Survey

An ill-considered library cut proposed last week would devastate an irreplaceable resource for the Earth sciences and for related industries, and should be vigorously opposed.

Times are hard at the United States Geological Survey (USGS). Two years ago, it escaped being abolished as part of the Republicans' anti-federalist and supposedly budget-balancing "Contract with America", only to come under renewed threat a few months later from a proposal — again from the Congress, and still pending — that all of the Department of the Interior's surveying and mapping activities should be handed over to the private sector.

But if the survey's mood is uncertain, its Geologic Division has had more immediate problems to face. With more research scientists than any of the other divisions, it has found it harder to justify all of its programmes as directly relevant to national needs. A forced staff reduction of 20 per cent in October 1995 has left its mark on division morale.

Even against this background, the recent decision to decrease the USGS library's acquisitions budget by almost 50 per cent (see page 637), and to give staff only one week to express views as to which subscriptions should be retained, stands out as cruel and unusual punishment. By common consensus, the library's journal holdings have already been pared to the bone over the past decade: duplicate subscriptions and titles readily available from other sources have long since disappeared. A cut of the magnitude envisaged would seriously jeopardize the quality of scholarship at the USGS, and thereby the quality of information that the survey can provide to the government.

By all accounts, the decision is more the result of conflicts between the survey's divisions than of strategic logic: the library sits within the Geologic Division, where most of the research is done, but the other divisions are charged according to their usage. At least one of the other divisions is said to have kicked up a fuss this year about the ever-increasing cost, and the result was the dramatic cut.

The repercussions, however, have been anything but parochial, and seem to have taken the survey administrators by surprise. For it turns out that they have been sitting on nothing less than a national

treasure — in the appropriate words of one former survey employee, a "master repository of the world's geoscience literature". University librarians throughout the country know the USGS library as the place where they can find material that cannot be found anywhere else. And librarians are not the only ones in the know. For example, after the collapse of the Soviet Union, a team of Exxon researchers were able to spend two productive weeks in the survey's library, assessing the geology of formerly inaccessible regions that had suddenly become attractive possibilities for exploration.

Viewed in this light, what seemed at first to be merely an inept management decision — inadequate in consultation and with insufficient appreciation of its significance — takes on the appearance of a national scandal. A resource that is not merely used but depended on by academic institutions, industry, regional agencies and the general public should not be at the mercy of an administrator trying to run one division of a troubled agency. People from outside the USGS account for almost half of the use made of the library, yet about 98 per cent of the library's funding comes from the survey's mission-oriented divisions, which have their own interests to promote. It comes as no surprise that such a mismatch has not been able to bear the strain of prolonged level funding or worse.

It is clear that the USGS library is, in all but name, a national library — akin to the Library of Congress and the national libraries of medicine, agriculture and education. Whether it likes it or not, the USGS is responsible for the future of this national resource. It should first remove the library from the Geologic Division, and acknowledge that maintaining a world-class Earth sciences library is part of the survey's overall mission of providing "Earth science in the public service". It should then ask the Congress to establish the USGS library as the National Library of Geoscience, funded explicitly as a line item in the budget. In this way, its wider role would be enshrined and, it is to be hoped, preserved from further attrition. □

More freedom in the Framework

The European Commission should have more independence in its research programme management.

Researchers familiar with the long delays in obtaining grants from the European Commission may not realize that one obstacle lies in the conflicting interests of the European Union's member states. In its newly proposed fifth Framework research programme (see page 639), the commission is asking for freedom to execute programmes without the constant supervision of political authorities. It suggests that its programme committees, which comprise national experts, should no longer "help" with the implementation of individual programmes, but should act only in an advisory capacity. This, it argues, will make decision-making smoother and quicker, and thus get money to researchers faster. And it has the support of the recent independent report by Viscount Davignon and colleagues on the running of past Framework programmes.

Member states are reacting aggressively to the idea, appearing not to trust the commission to deliver satisfactory results. But the interests of researchers have to be considered seriously, and vague distrust is not a sufficient reason to discard the idea out of hand. The burden of proof lies with the member states to justify their concerns.

The commission, too, has a burden of proof. Its proposal is too vague about its plans for a management structure that will need to be radically different to handle the complex matrix system it envisages for the fifth Framework. That is a justified source of concern for member states and for the European Parliament. All involved in the forthcoming decision process need to ensure that member states and the commission clarify their positions, in the interests of the effectiveness of researchers on whom they ultimately depend. □



Researchers find silver lining in delay to work on space station

[WASHINGTON] An announcement last week by the US National Aeronautics and Space Administration (NASA) of a delay of up to 11 months in construction of the International Space Station may have angered the project's supporters in Congress. But it is good news for scientists, as the agency plans to fly additional research missions on the space shuttle now that it will not be tied up delivering pieces of the station to orbit.

The delay is needed to provide time for a key Russian module to be completed. The Russian government has promised that money will be forthcoming to complete the service module, which will maintain the station in orbit and house its initial crew.

But NASA managers say they will not know until next month whether the Russians can keep the promise.

As a hedge against this problem, NASA has decided to let the date of the first construction flight slip from November 1997 to no later than October 1998. If Russia defaults, the United States would have to build its own 'interim control module' to keep the station in the proper orbit during the early stages of assembly.

Even before the slip in the schedule, NASA had been looking for ways to placate scientists who have become increasingly frustrated with the lack of flight opportunities for their orbital experiments in the near

future. To pay for cost overruns in building the station itself, the agency has had to defer development of some of the science equipment it is planned to carry.

Facilities once planned to be available for use by researchers in 1998 have now slipped to 2000 or later. At the same time, NASA has cut the number of remaining shuttle/Space-lab research flights to two — a multidisciplinary Microgravity Science Laboratory (MSL) which flew last week, and the Neurolab mission devoted to neurological research, which will carry an international payload in collaboration with the US National Institutes of Health next March.

This squeeze meant that researchers who depend on data from space experiments faced a long 'dry' spell, according to Martin Glicksman, a materials scientist at Rensselaer Polytechnic Institute in Troy, New York, and chair of a National Research Council committee that oversees NASA's microgravity research. He says that some disciplines were looking at a hiatus of nearly four years without experimental data. "People cannot be asked to stand in a queue that long."

Last week's premature landing of the MSL Spacelab mission — due to a failed electrical fuel cell — made matters worse. Astronauts were scheduled to spend 16 days in orbit, conducting 33 life science and materials science experiments and testing experimental hardware to be used on the space station. Instead, they came home after only four days, completing what little work they could by flashlight to conserve electricity.

NASA immediately announced plans to re-fly the MSL mission in July with the same experiments and crew. And now that the shuttle's short-term schedule has been freed by the station delay, the agency is considering additional flights in 1998 and 1999 devoted to research in life sciences, materials science and commercial product development.

Joel Kearns of the Marshall Space Flight Center in Alabama, who directs NASA's microgravity research programme, says the extra flights could either be reflights of existing MSL and Neurolab equipment, or newly developed equipment, which could fly either in Spacelab or in the privately leased Spacehab module. The agency hopes to fly some of the experiments it has already selected for the early station years on these shuttle flights instead.

The number of missions to be added, and how they will be financed, still has to be worked out between NASA, the White House and Congress. Money is scarce, particularly since the agency is asking

Canada swaps robot arm for lab space

[MONTREAL] Canada has agreed to build a vital component of the International Space Station in exchange for access to its laboratories under an arrangement announced after a meeting between the Canadian Prime Minister, Jean Chrétien, and US President, Bill Clinton.

Canada has already designed the Special Purpose Dexterous Manipulator (SPDM), which will handle small payloads such as batteries and computers, and operate small robotic tools for delicate maintenance tasks. But it was having difficulty finding the annual operating costs of about Can\$22 million (US\$15.8 million) required to use the space station's laboratories once they are in operation.

The deal is a trade-off, giving Canada a 2.3 per cent stake in the station's research capacity in return for reducing US construction costs, at a time when the space station's budget has been capped at US\$2.1 billion. But one of the reported implications is that Canada will have to withdraw from its associate membership

of the European Space Agency for several years.

Alain Poirier, the Canadian Space Agency's director-general for space systems, is responsible for the country's space station programme. He says that Canada had been given until this month to decide whether to provide the special purpose manipulator.

"If we had decided not to do so, NASA would have done it," says Poirier. "What was at stake was our leadership in space robotics." He adds that Canada also wanted "to use that to leverage our participation in the space station programme".

The manipulator is a successor to the 'Canadarm', used in the US space shuttle programme. It is a smaller, two-arm robot capable of the delicate assembly tasks at present done by astronauts during spacewalks. It will reduce the number of spacewalks required by providing the crew with telerobotic capability from inside the station.

Poirier says a large part of the C\$170 million needed to build SPDM will come from a contingency fund designed for new initiatives in Canada's

Long-Term Space Plan II, which covers 1994–2004. The rest will come from a reallocation of funds "to address new priorities".

He says it was "quite a challenge to fund a programme of the magnitude of the SPDM", but that the contingency funding has not been exhausted as a result. Nor will it be necessary to use funds from Long-Term Space Plan III for SPDM, as some had feared. That plan is still being formulated.

Ralph Nicholls, chairman of the Canadian advisory committee for the space station during its first five years, says he recognizes Canada's need to compete in space technology through the space station. But he says there is little in the programme for traditional space scientists. "There is a distinct lack of interest [among those in] space sciences, upper atmosphere research, space physics, space astronomy," he says.

According to Poirier, microgravity experiments have been funded under the second long-term space plan, but additional money for science projects will have to come from the third plan. **David Spurgeon**

Congress for an extra \$300 million to cover space station costs associated with the Russian delays. But members of the House of Representatives Science Committee were supportive during hearings last week.

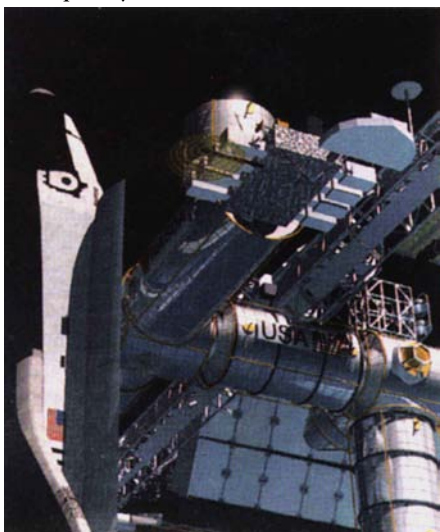
International space agencies have been invited to participate, says Kearns, which would help defray the \$100-million cost of a typical Spacelab flight. He expects a decision to be made in a month or two, after which the research community would be notified of any new flight opportunities.

The new flights would provide a short-term fix for space researchers, but there may be further troubles ahead. As space station managers dip into cash reserves to solve unforeseen problems, they may have to borrow again from accounts meant for developing science equipment.

In hearings last week, several members of Congress expressed concern that the science budget had been "raided" last year for nearly \$500 million. When asked if the money would be returned, NASA's head of life sciences and microgravity science, Arnauld Nicogossian, offered only vague assurances. "We believe there will be some payback of that money," he said, adding that the impact on his programme so far was "negligible" and that "we will be able to deploy most of the [science] facilities on time". That answer, responded Bud Cramer (Democrat, Alabama), "doesn't sound encouraging".

If Russia ends up defaulting altogether on building the service module, it would leave NASA without living quarters for the station's initial three-person crew. One contingency plan would be to make the US laboratory, due to be launched in 1999, habitable by adding facilities for sleeping, eating, and washing. That would mean some research facilities in the laboratory would have to be left off, to be replaced at a later date. The agency hopes it won't come to this, says one worried NASA manager. But "it's something to keep on eye on."

Tony Reichhardt



To boldly build... eventually: Delays in the space station will free up space shuttle missions.

Journals joust over policy on authors' interests

[WASHINGTON] A simmering dispute about the policies of journals towards the financial interests of their contributors erupted again this week with the publication in *Epidemiology* of an attack on the *New England Journal of Medicine* (NEJM) for its strict policy on this issue.

Epidemiology cited JoAnn Manson, an epidemiologist and endocrinologist at Harvard Medical School, as an innocent victim of the "police action" editorial policy of NEJM. Manson was publicly criticized by NEJM editors last autumn after she and Gerald Faich, a pharmacoepidemiologist at University of Pennsylvania Medical School, wrote an invited editorial in NEJM.

The editorial concluded that the benefits of the appetite suppressant dexfenfluramine (Redux) appeared to outweigh the risks from a rare but fatal side-effect, primary pulmonary hypertension.

Just before publication, it was revealed in press reports that Manson had previously acted as a consultant to the drug's makers, Interneuron Pharmaceutical and Servier Amerique, and that Faich had acted as an occasional consultant to Servier since 1994. Faich had also done consultancy work during the five months before the publication of the editorial for Wyeth-Ayerst, which markets Redux in North America.

At the time, NEJM forbade editorial authors to have "ongoing financial associations (including equity interest, regular consultancies, or major research support) with a company that produces a product (or its competitor) discussed in the editorial". (The policy has since been modified, with the words "ongoing" and "regular" dropped.)

In a subsequent editorial, NEJM executive editor Marcia Angell, and its editor-in-chief, Jerome Kassirer, wrote that the "disturbing" episode showed that "there is no better demonstration of the reason for our policy than the Manson and Faich editorial".

Even if the authors' conflicts of interest had been disclosed, they wrote, "troubling questions" would have remained for readers, such as whether Manson and Faich gave enough attention to nonpharmacologic treatments of obesity in reaching their conclusions. They wrote that Faich was clearly in violation of their policy, and would not have been allowed to publish had they known of his situation. Manson, they wrote, was in a "gray area" that required further discussion between her and the NEJM editors.

But, in next month's issue of *Epidemiology*, Manson defends her article as "cautiously written and... entirely independent of any influence by industry". She argues that

she and Faich had been "pilloried" by NEJM editors "over what were, in essence, misunderstandings". As a result, she says, "literally hundreds" of colleagues have expressed their support and their outrage at NEJM.

She adds that she considered it a "professional and civic duty" to assent when asked in 1995 by Interneuron and Servier to testify on their behalf to a Food and Drug Administration (FDA) advisory committee reviewing the drug. Manson presented her research on body weight and mortality in women and the health risks of obesity.

In an accompanying editorial, Kenneth Rothman, the editor of *Epidemiology*, and Cristina Cann, associate editor, write that the NEJM is "shooting itself in the foot" by refusing to publish editorials by authors with financial connections to their subjects.

Moreover, they write, the policy's "hopeless" goal of objectivity "debases the scientific process" by replacing substantive judgments with *ad hominem* evaluations, a process that is "inherently irrational" and risks "smearing honest scientists".

Angell dismisses the *Epidemiology* editorial as "confused fulminations", pointing out that neither it nor Manson's article deals with Faich's role as co-author at a time when he was doing consultancy work for Wyeth-Ayerst.

She says that Manson's *Epidemiology* article misrepresented the covering letter that accompanied the Manson-Faich editorial when it was submitted to NEJM. The letter said that "Manson and Faich participated in the FDA review of dexfenfluramine as scientific consultants on the health risks of obesity". "Do you see the mention of being a paid consultant for a company that stood to gain from the... sale of Redux?" asks Angell. "Is that a disclosure? I would think not."

Some experts say that appearances are so important that authors should disclose even one-off and financially minimal involvements. "In the public's mind — and certainly in the media's mind — it doesn't really matter whether or not the financial interests between an individual and a company were small or large," says Sheldon Krimsky, an expert in the study of scientific conflict at Tufts University in Medford, Massachusetts. "It's the principle" at stake.

But George Lundberg, editor of the *Journal of the American Medical Association*, rejects NEJM's "systematic exclusion" of financially interested editorial and review authors. He says that his journal's policy would have allowed "serious consideration" of publication of the Manson-Faich editorial with full disclosure.

Meredith Wadman

UK astronomers hit out at funding council

[LONDON] Britain's astronomers are losing confidence in the Particle Physics and Astronomy Research Council (PPARC), according to a survey of members of the Royal Astronomical Society. Their main concern is the council's perceived failure to protect the existing ground-based astronomy programme from a £7-million (US\$10.5-million) funding cut that could lead to the closure of up to three telescopes to which Britain is a major contributor.

The savings are designed to help support the UK contribution towards the new Gemini project, two 'next-generation' eight-metre telescopes being planned jointly with the United States, Canada and countries of South America.

Summarizing the survey's findings at the start of the UK National Astronomy Meeting in Southampton last week, Malcolm Longair, president of the society and formerly Astronomer Royal for Scotland, said there was a feeling that PPARC could have managed the issue more effectively.

"There is considerable concern that PPARC has allowed the current crisis facing the ground-based astronomy programme to come about so suddenly so far as the wider community is concerned," said Longair.

But Ken Pounds, chief executive of PPARC and professor of astronomy at the University of Leicester, says that a succession of "funding squeezes" has left PPARC with little choice but to withdraw funding from

two telescopes on La Palma, in the Canary Islands, as well as the UK-Schmidt Telescope in Australia. "We are going to have to make reductions to the size of the existing programme, for if we don't invest in new things, we will get left behind."

In January, a PPARC committee recommended that most of the £7-million savings should come from the Isaac Newton Group of telescopes that are part of the Royal Observatory network but situated on La Palma.



Pounds: cuts leave little choice.

Longair says there is "broad agreement" on the need to reduce Britain's existing ground-based astronomy programme to operate the new Gemini telescopes. But he says there remains "a strong expression of feeling that the current drastic round of economies being required is extremely damaging". He also says the lack of effective communication between PPARC and the astronomy community meant that many astronomers did not understand why the cuts had to be made.

Steve Unger, director at the La Palma site, is among those who believe that PPARC's latest proposals are unfair and shortsighted. Unger says that he was already implementing a 20 per cent "efficiency saving" following an earlier attempt at finding ways of diverting money into Gemini, a review panel

that was chaired by James Hough, head of the department of physical sciences at the University of Hertfordshire.

Unger says he is now being asked to find an additional 15 per cent, which would effectively cripple a facility that in recent years has produced more highly cited papers than its Royal Observatory stablemates. He also believes that PPARC's plans to close one of the La Palma telescopes — the Jacobus Kapteyn Telescope — will be largely symbolic, as the "cost of operating the telescope is very much less than the cost of demolishing it".

Astronomers trace part of PPARC's difficulties to a shift in government emphasis from 'big physics' projects towards the life sciences. "There is no question," says one senior astronomer, "that funding for physics and astronomy has fallen by two to three per cent per year over the past 10 to 15 years."

PPARC's problems, he says, can also be attributed to the 1993 White Paper on science and technology, which led to the break-up of the old Science and Engineering Research Council (SERC), and the emergence of PPARC and a separate council for engineering and physical sciences.

"Before the White Paper, when funding was tight, astronomy and particle physics could look elsewhere within the old SERC for money that had not been spent. This is now impossible, and astronomy has no equivalent of the Wellcome Trust." **Ehsan Masood**

Copyright treaties threaten information flow, says NRC

[WASHINGTON] A committee of the US National Research Council (NRC) — the executive arm of the National Academy of Sciences — last week repeated its warning that international treaties now under consideration to govern copyright law for electronic databases could hamper the free flow of scientific information.

"The scientific community and its defenders must step in quickly to insist on further, open debate before these changes reach implementation," says the final report of the NRC Committee on Issues in the Transborder Flow of Scientific Data.

The committee, chaired by Stephen Berry, a professor of chemistry at the University of Chicago, began studying data policy issues in 1995. Last year it took the unusual step of releasing one chapter of its report early, immediately before a diplomatic conference on copyright treaty held by the World Intellectual Property Organisation (WIPO) in December. Several scientific organizations have argued that giving copyright to compilers of electronic databases would lead to researchers being

charged for data they at present receive free (see *Nature* 384, 299; 1996).

WIPO representatives and others say these fears are overblown. But opposition was strong enough before the December conference for discussion of electronic databases to be dropped. The subject will be taken up again at an "information meeting" to be held by WIPO in September.

The NRC report released last week repeats the call for any new treaty to include "fair use" exceptions that would allow scientists and educators to use copyrighted databases either free or at reduced cost. The committee argues that the free and open exchange of scientific information is a "public good" that needs to be protected.

With this guiding principle in mind, government science agencies eager to cut their operating costs should be careful about privatizing the distribution of research data, says the committee. Those who contribute to a database — the scientific community — should be able to use it free.

If a commercial distributor adds value to the raw data, the price for researchers and

educators "should be no higher than the marginal cost of adding value". For their part, "all scientists conducting publicly funded research should make their data available immediately, or following a reasonable period for proprietary use".

When deciding whether to privatize a database, whether it contains Earth satellite imagery or information on the human genome, government agencies should consider such factors as how large and diverse the user community is, and whether the market can sustain more than one data provider. If there is no market beyond scientists, says the NRC committee, it makes sense for the government to continue to distribute the data.

The committee recognizes that some of the current conflict over copyright law stems from different use of the Internet — businesses tend to protect information, but scientists want it exchanged openly. One possible solution would be for scientists to create their own international science network along the lines of the Internet II now being developed. **Tony Reichhardt**

Budget cuts force Sweden to consider CERN pull-out

[MUNICH] Sweden's particle physicists are trying to persuade their government not to withdraw from the European Laboratory for Particle Physics (CERN) in Geneva, Switzerland, to make savings in Sweden's 1998 research budget.

Withdrawal from CERN is one of the options being considered by a special commission set up earlier this year by the Swedish ministry of education, which is responsible for spending on science, to study ways of cutting SKr150 million (US\$19.5 million) from its budget for large-scale international research collaborations.

The cuts are being made as part of efforts to bring the Swedish economy in line with the so-called 'Maastricht criteria' that will make the country eligible to join a full European economic and monetary union.

Last autumn, Carl Tham, the education and research minister, announced cuts averaging 15 per cent to the budgets of Sweden's national research councils in 1997, in a move intended to save about SKr300 million a year. Tham said that Sweden's subscription to large international research programmes should bear the burden of further cuts of SKr150 million expected for 1998, and that collaborations with international laboratories — such as the European Molecular Biology Laboratory in Heidelberg — should be closely scrutinized.

Tham appointed Susanne Eberstein, a member of the Swedish parliament with expertise in research policy, to carry out this task. Her report is due to be published at the end of this month.

Drawing on advice from research funding agencies and others, Eberstein has identified complete withdrawal from CERN as a possible way of achieving the planned cuts. She says it is not possible to spread the reductions among different international laboratories, as subscriptions are determined by the gross national product of member states.

This solution also has a financial tidiness because Sweden's annual subscription to CERN — which at SFr27 million (US\$18.5 million) comes to 3 per cent of the laboratory's total budget — is approximately the size of the planned cut.

As a possible alternative to cuts in the international arena, Eberstein is also considering spreading the cuts between national large-scale research activities.

Swedish scientists are campaigning vigorously against withdrawal from CERN. The Swedish Physical Society has been leading a letter-writing campaign to draw attention to the importance to Swedish scientists of continuing membership.

Per Carlson, a professor of particle physics at Stockholm's Royal Institute of Technology, says that membership is of vital importance to Sweden's small particle physics research community. "It would be a scandal to leave CERN at a time when countries like Poland, Hungary and Slovakia are increasing their membership fees to CERN."

On the basis of the recommendations of the Eberstein report, the government is expected to decide in September how to implement the cuts in the 1998 research budget.

Alison Abbott

French law would help scientists to go into business

[PARIS] The French government is planning to introduce legislation that would encourage government-funded scientists to create their own companies and cooperate more with industry. The law will modify the status of researchers as civil servants in order to allow them to work in the private sector.

The proposed law will also allow government scientists to work in a small company for four years while leaving open the possibility of returning to their previous posts. This period would be taken into account in staff evaluations. The researchers would be allowed to own up to 10 per cent of the equity of private companies, and to act as consultants.

Announcing the plans, which have already been approved by the French cabinet, François d'Aubert, France's minister of research, said that scientists were one of the main factors in encouraging economic growth and job creation. "We must give them an opportunity to create companies without any danger to their career, or any conflict of interests," he said.

D'Aubert urged research organizations to create specialized units to support researchers wishing to set up companies. He suggested that scientists starting such ventures should be able to use their laboratories as company premises, provided they paid a "very low rent". He also said that the new rules on equity ownership would bring French practice in line with that in the United States.

The ministry is planning to set up a venture capital fund to support companies created by scientists, in a bid to make up for the relative lack of such funding available in France. But d'Aubert gave no details as to when this fund will be launched, or its size, saying only that it would be funded equally by the public and private sectors.

How successful the initiatives will be in stimulating the creation of companies remains an open question. According to a study by Philippe Mustar, of the Ecole des Mines de Paris, scientists from the atomic energy commission (CEA) set up fewer companies between 1988 and 1991 than researchers from the national research agency, CNRS, even though they do not suffer from the constraints of being civil servants.

And the demand to set up companies is likely to remain low, in the near future at least. According to one CNRS official, only about ten researchers each year voluntarily ask for information about establishing their own company.

Eric Glover

Body work leads to UK sculptor's arrest

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UNAVAILABLE
FOR COPYRIGHT
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[LONDON] A British sculptor and a former employee of the Royal College of Surgeons have been arrested and bailed in connection with the removal of the remains of human corpses intended for medical research. The arrests coincided with the discovery by

police of parts of the bodies of up to 30 people at houses belonging to Anthony-Noel Kelly (pictured), a sculpture tutor.

An exhibition of Kelly's work earlier this year included a cast of a silver-coated man with parts of his brain cut away. □

Library cuts bring tremors to geologists

[WASHINGTON] The US Geological Survey (USGS) is reassessing the role of its library, which is increasingly being used as a national information resource, following widespread criticism of a threatened cut to the library's acquisitions budget.

At the end of March, P. Patrick Leahy, the survey's chief geologist, announced to survey employees that, for fiscal year 1997 (starting on 1 October), spending on periodicals would have to be reduced to about \$422,000 — little more than half the amount (\$815,000) required to continue the 2,238 subscriptions currently held by the library. (The book budget would be reduced by the same proportion, from \$150,000 to \$78,000.)

But last Thursday (10 April), Edward C. Roy Jr, president of the American Geological Institute (AGI) — which represents 30 member societies in the Earth sciences — capped a week of protest from both inside and outside the survey by sending a letter to the survey's director, Gordon Eaton, expressing "strong opposition" to the deep funding cuts.

Scientists at the Geologic Division — which employs most of USGS's research scientists and provides more than three-quarters of the library's funding — have been angered by the scale of the budget reduction, and by the fact that they were given only a week to suggest which journal subscriptions should be retained. A letter to Leahy from 55 scientists at the survey's headquarters in Reston, Virginia, expressed "horror and disbelief" at "this self-destructive decision".

Opposition to the proposed cuts from outside the survey has been led by Earth science librarians in universities, who view the USGS library as an invaluable information resource, especially as a source of foreign and other 'obscure' publications.

Having been forced to cancel subscriptions in response to their own budgetary pressures, librarians elsewhere in the country have come to rely on interlibrary loans from the USGS library. Dennis Trombatore, of the Walter Geology Library at the University of Texas at Austin, described the library holdings as "the jewel in [our] crown... the place of last resort for unusual materials", and "the best Earth science library in the world".

Another cause for alarm is that the Reston branch of the USGS library serves as the most important single resource for the GeoRef bibliographic database, and its printed counterpart, the *Bibliography and Index of Geology*. This database, produced by the AGI in Alexandria, Virginia, contains more than two million references to all types of Earth science information, including maps and theses as well as journal articles and books. It extends back to 1785 for North American geology, and to 1933 for the rest of the world.

John Mulvihill, GeoRef director at the

AGI, explains that the institute cannot afford to subscribe to all of the 4,000 or so publications currently indexed in GeoRef. Although the database receives bibliographic information from the journals themselves, it uses the Reston library to verify this information, and to acquire more detailed information for indexing.

In his letter to Eaton, Roy says GeoRef is already "scrambling to compensate for the subscriptions lost from earlier cuts [to the Reston library]" — 1,667 subscriptions have been cancelled since 1986. Charlotte Derksen at Stanford, who is on the GeoRef advisory committee, considers the Reston collections to be "the backbone and the flesh" of GeoRef, and suggests that the result of the proposed cuts "could well be [its] demise".

According to Barbara Ryan, USGS associate director for operations, the decision to cut the library budget so dramatically reflected disagreements between the different divisions of the survey. The library is part of the Geologic Division, but, with other divisions having to produce money for the library, "it's been a contentious issue for some time".

Last Friday (11 April), the library budget came up for discussion by the survey's policy council, whose members include the director, associate directors and heads of the USGS divisions. After the meeting, Ryan said that the reaction to the proposed cuts, and in particular the fact that the library was an important resource for users outside the survey, had prompted the policy council to reconsider the role of the library and how it



should be funded.

"Is it a library that just supports our research? Or a world-class collection that needs to be managed as such?" She said these questions were being put to the Library Board of Governors, a newly appointed body that met for the first time last month.

The board of governors was not consulted about the proposed cuts, according to its chair, Beth Duff. "The paperwork was in the mill at the time we met." The board is expected to report on its deliberations this autumn.

Meanwhile, there is the question of this year's budget. Ryan suggested there might be some flexibility — "we may take [the total for books and periodicals] up to \$700,000" — but said that finding the extra money would be the responsibility of the Geologic Division. In the longer term, Ryan suspects that moving the library out of the Geologic Division, into the survey-wide Office of Program Support, will save it from becoming an object of interdivisional warfare.

Laura Garwin

Medical centre repays 'overbilled' \$15.5m

[WASHINGTON] New York University (NYU) Medical Center agreed last week to repay to the federal government \$15.5 million in connection with alleged overbilling of indirect research costs. A government spokesman said that four other universities are being investigated about similar allegations, but he refused to name them.

The NYU sum is the largest ever repaid to the government, and the House of Representatives Commerce committee, which has jurisdiction over indirect costs, is in contact with the Department of Health and Human Services and is "reviewing the situation". It was this committee, now chaired by Thomas Bliley (Republican, Virginia), which, under John Dingell (Democrat, Michigan), conducted widely publicized hearings on Stanford University's overbilling of indirect costs in the 1980s.

The NYU Medical Center's agreement to pay follows a three-year investigation by federal officials. The government concluded

that the university had provided a "false, inaccurate and incomplete picture" of its research overhead costs in 1982 and 1993. For example, it said, the university billed food for medical school commencement ceremonies as research overhead costs.

The medical centre said it agreed to the settlement only in order to avoid a lengthy legal battle. "We clearly made some accounting and administrative mistakes," it said in a statement. But these were "inadvertent", and it continued to disagree with any characterization that "might suggest intentional misconduct".

Mary Jo White, the US Attorney for the Southern District of New York, said: "They intentionally submitted false claims in order to receive more money than they were entitled to." Cornelius J. Pings, president of the Association of American Universities, urged that the case "not be seen as evidence of a need for destabilizing a system that, on the whole, works well".

Meredith Wadman

Fishermen fight rangers in conservation battle for Galápagos

[MUNICH] Violence has broken out in the once tranquil Galápagos Archipelago as tensions intensify between illegal fishermen and conservationists intensify.

In the most serious incident, fishermen opened fire last month on park rangers who had found their illegal camp, seriously wounding one of them. In other incidents, park rangers, local politicians and the captain of a patrol boat have been attacked with cudgels and bottles, and threatened with death.

The islands have appealed to the government of Ecuador, to which they belong, for support from the navy to protect staff. Robert Bensted-Smith, director of the islands' Charles Darwin Research Station, says that the violence, though serious, "is confined to a small, hard-core group of fishermen who are taking an aggressive stand".

The Ecuadorian islands retain 90 per cent of the biodiversity that was observed by Charles Darwin when he visited them in 1835. But although they have been designated as a national park, and listed as a

World Heritage Site by the United Nations Educational, Scientific, and Cultural Organization (UNESCO), management of their ecological resources has become increasingly difficult.

Their waters, including the supposedly protected marine reserve, have been seriously overfished in recent years. Limited fishing quotas, and the total ban on the fishing of the sea cucumber, *Isostichopus fuscus*, a coveted delicacy in Asia that is threatened with extinction (see *Nature* 383, 3; 1996), continue to be ignored.

The fishermen pose a serious threat to the biodiversity of the world's most valuable ecological resource, not only by depleting it of important marine species, but also by introducing new species such as rats and innumerable insects which upset the archipelago's ecological balance.

The islands are also threatened by increased immigration from poorer provinces of Ecuador. A controversial bill introduced by a local politician last year would have controlled this immigration, but would have allowed immigrants a level of

agricultural activity that conservationists believed would also threaten the ecological balance. The bill was abandoned when the shaky Ecuadorian government fell earlier this year, and the new government has set up a committee to redraft it. Bensted-Smith is optimistic that the new version, which the government hopes to present to parliament in June, will be more favourable to conservationists.

This is partly because the conservationists' cause now has an additional, and powerful, political weapon, he says. UNESCO's World Heritage Committee last December placed the Galápagos Islands on its list of World Heritage Sites in Danger. This means that the Ecuadorian government has until November to offer persuasive arguments that it will be able to contain the threats to the islands' biodiversity.

If it does not, UNESCO will strip the islands of their status as a World Heritage Site, a move that would inevitably damage the tourism upon which the Galápagos' new-found wealth depends. **Alison Abbott**

'Ark evidence' challenged in Sydney court

[SYDNEY] It was hard to find standing room inside Federal Court 23C as the now world-famous 'Noah's Ark/Creationism Trial' opened in Sydney, Australia, last week.

Ian Plimer, a geologist at the University of Melbourne, and David Fasold, former marine salvager of Oregon in the United States, are alleging that Allen Roberts engaged in misleading and deceptive commercial conduct and breach of copyright. In 1992, Roberts, a Bible Church elder and self-styled historian and archaeologist of Sydney, claimed to have "scientific" evidence for the remains of Noah's Ark in Turkey and raised funds for an "expedition" (see *Nature* 386, 529; 1997).

Early attempts to confine the court proceedings to commercial aspects were eroded as Plimer wove into evidence statements on academic standards and responsibility, and the methods and philosophy of science. Judge Ronald Sackville, former dean of law at the University of New South Wales, intervened to clarify each of these areas on the record. A judgement, however, can be made only under laws on trade practices and copyright.

When asked about Roberts' doctorate in Christian education from Freedom University, a correspondence Bible college that moves around Florida and which Roberts admits is not accredited, Plimer said: "I would not have the gall to display it behind my name and call myself an educator."

Alex Radojev, senior counsel for Roberts,

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Fasold: believer
turned critic.

ence and misleading to young people.

Plimer labelled Roberts "a new creationist on the block" and declared that, as a university professor, he has "duties and responsibilities to the people [at large] who fund me".

Plimer studied the site near Mount Ararat in 1994 with Fasold, who was then still an Ark believer, and was recorded by television crews and magazine journalists. Plimer rejected Roberts' claims that "petrified wood", "rivets", "slag" and "drogue" (anchor stones) were common to the site, and said there was no evidence for "metallic" material, "uplift" following an earthquake or a "Great Flood". Plimer said the "mud" covering much of the structure is modern.

In evidence, Plimer said that a Turkish geophysicist from Ataturk University, Salih Bayraktutan, who has virtual control over the site, had prevented any excavations by Plimer, saying he "supported the views of Christian fundamentalists that this is the site of Noah's Ark".

But, according to Plimer, Bayraktutan

probed Plimer's knowledge of the alleged Ark site and queried the "credit" of his motives in bringing the case. Plimer responded that he was "affronted" by each of Roberts' claims of "evidence" for the Ark as being unfounded in science.

said he didn't believe in Noah's Ark and that this was his equivalent of Loch Ness, adding "I'm sorry. This is the only way I can fund my research". Plimer has reported him to the rector of his university.

Fasold had depended heavily on Bayraktutan's information during several visits to the site and for a book he had written. Outside the court, Fasold said this was the first time he had heard of Bayraktutan's statement, but it explained how he had been misled for so long.

In evidence, Fasold said he was misled by John Baumgardner, a geophysicist at Los Alamos National Laboratory, New Mexico, who visited the site with him in 1986 and claimed his tests confirmed the authenticity of the remains. Fasold said that when he found last year that Baumgardner is a "creation scientist", his faith in the site collapsed.

As Roberts' barristers found difficulty in handling scientific evidence, the judge ensured the court was treated to lessons in Karl Popper's theory that science advances by successive falsifications, Lamarckian inheritance, Darwinian evolution and academic credentials.

Radojev tried to pin Plimer to a yes/no answer on the argument by creationists that evolution is unproven "theory" and not "fact". Plimer countered by repeating, until his interlocutor gave up, that evolution is "a process" drawing on interdisciplinary science. **Peter Pockley**

European research programme faces rough ride ahead

[MUNICH] The European Commission last week formally published its proposals for the fifth Framework research programme (FP5), which will last from 1998 to 2002. But the proposals face a rocky ride in winning the necessary approval of the Council of Ministers and the European Parliament.

The proposals do little to clarify the details of the proposed content of the programme, nor the details of how the complex management arrangements will be handled. The commission is keen to remain flexible on these points until it has the approval of member states for the general direction of its planned programme.

But member states are expected to withhold their approval until more details are presented. They are also expected to ask for substantial modification of the proposals. Another contentious issue is the level of influence member states will have in the running of the programme.

One new element in the formal proposals on FP5 is a statement that the commission will fund biological research only if bioethical issues have received appropriate consideration. The commission says that it will not support work on germ cell therapy or the cloning of humans. Cloning or genetic alteration of animals will be approved for funding only under ethical guidance, including respect for both animal welfare and the principle of genetic diversity.

China plans large-scale science projects

[TOKYO] China has announced plans for four ambitious science projects — an advanced sky survey telescope, a national synchrotron radiation laboratory, a seismic monitoring network and an advanced light source — to be constructed before the end of the century at an estimated cost of 2 billion yuan (US\$240 million). According to the Xinhua news agency, the telescope, said to be the most advanced of its type in the world, will be built in the Yanshan Mountains of north China, and should be capable of observing 100 million galaxies.

The Chinese Academy of Science is planning to construct eight new light beams and eight laboratories for the National Synchrotron Radiation Laboratory at Hefei in eastern China's Anhui Province. It will also collaborate with the city government of Shanghai to build a new-generation synchrotron light source, which will operate at between 2.2 and 2.5 GeV. A total of 120 million yuan will be used to build the seismic monitoring network using a global satellite positioning system.

Physicist plans 'power of prayer' experiment

[LONDON] Six hundred patients awaiting heart surgery in US hospitals are to take part in an experiment to test whether prayer has an effect on their symptoms. The idea comes from Russell Stannard, professor of physics at the Open University in England, and a practising Christian.

Two groups will be told they may be prayed for, one of which will be prayed for, and a control group which will not. Patients in a third group will be made aware that God is being asked to heal them. The Templeton Foundation is funding the two-year project.

Parliament calls for labels on modified maize

[MUNICH] The European Parliament last week called for Ciba's controversial genetically modified maize, which was authorized for import and sale in the European Union (EU) by the European Commission last December, to be labelled as such. A new EU rule requiring compulsory labelling of genetically engineered seeds, which the commission approved earlier this month (see *Nature* 386, 532; 1997), does not apply retrospectively.

At the same time, Gen-ethisches Netzwerk (GeN), a group of German activists opposed to genetic engineering in agriculture, says that resistance to field trials of genetically modified crops will continue.

Senators back increase for Parkinson's funds

[WASHINGTON] More than one-third of US senators are backing a bill calling for a tripling of funding for Parkinson's disease research. The Morris K. Udall Parkinson's Research and Education Act, introduced in the Senate last week, would authorize \$100 million in National Institutes of Health (NIH) funding for research on the disease in 1998, establishing up to ten research centres.

The NIH has consistently resisted such 'earmarking' of funds. Last year, responding to a bill introduced in 1995, its director Harold Varmus said the NIH could not "responsibly" make use of such a huge infusion of funds for Parkinson's research. An estimated one million Americans are afflicted by the disease.

Mathematician pursues clean copyright case

[LONDON] Sir Roger Penrose, professor of mathematics at the University of Oxford, is preparing to take the maker of a leading quilted toilet tissue to court for breach of copyright. Penrose alleges that the company Kimberly Clark, owner of Kleenex, copied his 20-year-old aperiodic pattern of distinctive

star shapes, which he (and apparently the company) believes gives the lavatory paper its distinctive softness and feeling of bulk. He is demanding a share of the profits, and that paper and documents containing his copyright pattern be returned to their rightful owner, or destroyed.

Carnegie man tipped to take over at Scripps

[WASHINGTON] Sean Solomon, director of the department of terrestrial magnetism at the Carnegie Institution of Washington, is being tipped to take over as director of the Scripps Institution of Oceanography in La Jolla, California. Tom Jordan, head of the department of Earth, atmospheric and planetary sciences at Massachusetts Institute of Technology, is understood to have been shortlisted for the post along with Solomon. But negotiations with Solomon, a widely respected geophysicist, have been going on for three months, which suggests that he is now the sole candidate. Neither Solomon nor Scripps is prepared to comment, and an announcement is expected shortly.

Sixteenth-century sphere hits heavenly price

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[LONDON] A 400-year-old brass model showing the Earth as the centre of the Universe and built for an Ottoman sultan with a passion for astronomy was sold in London last week for £771,500 (US\$1.2 million), three times the expected price. The Ptolemaic armillary sphere (above), which is 16 inches high, is believed to have been made in 1579 by craftsmen in Flanders.

The brass rings depict the motion of celestial bodies around the Earth and include one for the 12 astrological 'houses of heaven'. Sultan Murad III's fondness for astronomy was not shared by some of his more orthodox religious advisers; an observatory built by the sultan attracted religious opposition, and was destroyed in the year it was built following the imposition of a *fatwa*, or edict.

Harmonizing European patent practice

Sir—The view of Howard Dalton *et al.* that the proposed European Directive on the Legal Protection of Biotechnological Inventions “poses a threat to the future of scientific and medical research” (*Nature* 385, 672; 1997) is based on several misconceptions. The directive seeks to harmonize European patent practice in biotechnology; it does not add any new types of patent practice, nor does it broaden what is and what is not patentable in biotechnology.

Patents are granted to inventors to protect their inventions. In exchange for disclosing the invention to the public, the inventor has the right to prevent others from commercializing the invention for a limited period in a specific geographical area. Through disclosure by publication, patents actually reduce secrecy in scientific research. The research exemption provision, moreover, allows experimentation on a patented invention without infringement.

Several hundred European patents covering biotechnological materials, whether nucleotide sequences, proteins, cell lines, antibodies or transgenic organisms, and the processes involved in their production, have been granted by European patent offices over the past fifteen years.

These materials and processes are not automatically patentable simply because they are novel. Unlike scientific discoveries and mathematical equations, patentable inventions must have an “inventive step” and be industrially applicable. It is simply not true that a company has only to make sure of a “microbiological process” to obtain patents. Current European patent law expressly excludes biological materials derived from “essentially microbiological processes”.

Nor does the directive take further the question of claim scope. The Biocyte patent has been examined by the European Patent Office according to its patenting criteria. For those who consider this to be the wrong decision, there are established mechanisms to challenge such patents. In general, the scope of biotechnology patents has been getting narrower in Europe. This is a natural consequence of the evolving case law that surrounds biotechnology patents.

It is the responsibility of companies to develop, test and market new drugs and diagnostics. Without the protection offered by patents, they will not make costly and often high-risk investments in new genetic technologies. Although medical charities have raised millions for research to discover the causes of genetic disease, they cannot

possibly fund the development of new treatments. Nor should they. Patient support groups are demanding that the benefits of increased scientific understanding are translated into effective diagnostics and treatments quickly, safely and efficiently. The harmonizing proposals within the directive will be an important element in meeting these entirely legitimate concerns. The only alternative to patent protection is secrecy, which will impede research and encourage research duplication.

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Patronomy pioneers

Sir—Thomas Brooks recently reported on the remarkable case of the newly discovered bird *Vireo masteri*, whose name has been auctioned by BirdLife International (*Nature* 385, 574; 1997). He welcomed this and proposed patronomy as a general strategy for worldwide taxonomy. The Zoologische Staatssammlung München (ZSM) has been doing this for more than a year.

The discovery of the biosphere, the documentation of fossil or extant organisms, is one of the most pressing needs of our time. But, whereas ‘Systematic Agenda 2000’ is well known in the United States, the public in Europe, and in Germany in particular, is only now becoming aware of it. After decades of cutbacks, biosystematic institutions will, without help, certainly fail to fulfil the actual requirements. ‘Systematic Agenda 2000’ needs partnership. The potential partners, of course, want something for their money, and we can offer patronomies as small but eternal monuments.

The ZSM, a public research institution of zoological systematics, does the following: after a donation to our supporting society (DM5,000 for individuals, twice as much for

institutions) and the selection of ‘his/her’ animal, the donor gets a document confirming the patronomy, formal confirmation of the donation (which can be offset against taxes), an original graphic of the animal (a copy is published), and ten reprints of the published paper. Documents and graphics are presented at celebration events twice yearly.

Although the dedication of a species name “just for money” may be disagreeable to some people, I regard this as a matter of necessity, and its introduction overdue. The idea is hardly new. Many classic names of species were dedicated to express thanks and to honour the (financial) support of research projects, and to encourage the patron to continue making donations. Within our project, the scientific author also thanks the donor for support of biosystematic research.

Clearly, there must be rules to prevent misuse of the project. Individual scientists are encouraged, but not obliged, to take part, and both the ZSM and the systematists are free to refuse sponsorship. The statutes of the supporting society, Freunde der Zoologischen Staatssammlung, guarantee the administration of the donations for zoological research, and the money is spent

to buy collections or books, to support scientific journeys or to finance urgently needed equipment.

The patronomy project has been running at ZSM for more than a year. Some of the results have been unexpected. Reports in the press and on television are creating rapidly increasing interest among potential donors, and the media have reacted positively and with enthusiasm. Through these reports, we have also been able to increase public understanding of the problems of biosystematics and biodiversity research. In my view, increasing public awareness is a prerequisite to having a voice in politics. The donors (13 so far) are members of the general public who usually want to dedicate an animal to a loved person. Conventional dedications (*‘honoris causa’*) should not, of course, be replaced but supplemented by the project.

The first volume of papers of the project, ‘Patronomies’, has been published (*Spixiana*, Supplement 22) and a second series will appear in late autumn.

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Learning from history

Sir— Your leading article advocating a moratorium on human cloning¹ places the Berg letter, advocating a moratorium on recombinant DNA research, in 1976, and states that it “was soon followed by strict regulation” — two serious inaccuracies. Given that we are still running the same debate, at least in Europe, a quarter of a century later, *Nature* should get it right.

A biohazards conference was held at Asilomar, California, in February 1973, and in June that year the annual Gordon Conference on nucleic acids, held in New Hampton, New Hampshire, was also devoted to hazards in rDNA research. The co-chairs of the latter meeting addressed a letter to the National Academy of Sciences (NAS) and the Institute of Medicine, requesting the formation of a study committee to assess the hazards and recommend appropriate action². NAS appointed Paul Berg to chair the resulting study committee; its report was published in *Nature* and *Science* in July 1974 (ref. 3). It called for voluntary deferment of certain experiments, pending further research; for the National Institutes of Health (NIH) to establish an advisory committee to develop guidelines; and for an international

meeting, which took place in February 1975 at Asilomar.

Donald Fredrickson, the director of NIH, established the NIH rDNA Advisory Committee immediately afterwards, its first guidelines being released in June 1976. These were mandatory for NIH-supported research, and followed voluntarily by others. Contemporary demands to legislate for “strict regulation” were finally seen as unnecessary in the congressional debates over the following months and years.

Again, the parallel debate in Europe was interesting: the strict containment directive proposed by the European Commission in 1978 was in 1980 replaced by what was adopted as Council Recommendation 82/472 in 1982, advocating national registration of such research, and regular review over subsequent years in the light of experience with the conjectural hazards — an admirably pragmatic approach, now sadly abandoned. The United Kingdom’s responses in 1974–76 were similarly practical — the Ashby working party set up in the month of the Berg letter reported by December 1974 (ref. 4), in time to influence the debate at Asilomar; the Williams working party set up thereafter published guidelines in August 1976 (ref. 5), allowing research to recommence.

After ten more years of debate and experience, the council of the Organization

for Economic Cooperation and Development could conclude “that there is no scientific basis for specific legislation to regulate the use of recombinant DNA organisms”⁶.

All of which confirms the empirical observation that “those who do not learn from history are condemned to repeat it”.

Mark Cantley

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Deserving of trust

Sir— The News article “UCSF settles lawsuit over research costs” (*Nature* **385**, 377; 1997) raises the unfair implication that the Ischemia Research and Education Foundation (IREF) is a mere shell created and manipulated by Dr Dennis Mangano to

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PCR results means
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Terri Davis is a cellular
biochemistry technician
working in New York, NY.



appropriate research funds.

In fact, IREF is a nonprofit public benefit corporation directly employing 60 people that works with more than 300 medical and scientific researchers participating in the Multicenter Study of Perioperative Ischemia (McSPI) Research Group in 160 clinical and research facilities worldwide. This unique organizational structure, in which hundreds of institutions and researchers share sensitive medical outcome information with an unbiased, independent nonprofit research facility, has made it possible for McSPI and IREF to overcome academic and competitive rivalries while accommodating legitimate concern about confidentiality to gather and analyse empirical data on a very large scale, with extraordinary results.

With the data and resources IREF has made available in the past three years alone, McSPI investigators have submitted more than 80 abstracts and manuscripts, including two recent lead articles in *The New England Journal of Medicine* (5 and 19 December 1996) and the *Journal of the American Medical Association* (22 January 1997). One of these studies demonstrated that an inexpensive generic beta-blocker could supplant far more costly drugs being developed by the pharmaceutical industry and save more than 50,000 lives a year among patients undergoing complex

surgery. Another IREF-sponsored epidemiological study showed that the risk of neurological damage following coronary artery bypass graft surgery was significantly higher than had previously been accepted in the medical community.

At a time when the cost and quality of medical care are of paramount concern, and when more than 30 million surgical procedures are performed annually in the United States alone, the public needs continued access to the results of our epidemiological studies. To continue this work without further distraction or expense, IREF decided to settle the lawsuit concerning the University of California, San Francisco. We have been and remain deserving of the public trust and the trust of the many scientific investigators participating in IREF's good works.

Ahvie Herskowitz

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Unequal letters

Sir — Maybe I am missing something in the recent thread of letters about varying citation frequency depending on an author's alphabetical position. In case I am not, I should like to point out another

possible explanation: there are more people with surnames starting with letters in the first half of the alphabet, hence the higher frequency of citations.

From looking at the telephone book for Hamburg, Germany, which is divided into two volumes, A–K and L–Z, it is obvious that names starting with the first eleven letters of the alphabet are more common than all the others, with the exception of 'S', which is the single most common first letter. Depending on differences in ethnic and national distributions of first letters in names, there should not be an even distribution of citations, even if all potential authors had an even chance of being first authors. Yet another explanation that has not been mentioned as far as I recall would be a preference of people whose names start with letters from the beginning of the alphabet for research — either by their own choice of career or through bias in hiring.

K. Thorsten Jaekel

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● **When the London telephone directory was published in four equal parts (until a few years ago), the first section was A–D.**

— Editor, *Nature*.

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Problems with stockpile stewardship

US government proposals for maintaining competence in nuclear weapons while observing a test ban have serious flaws. An approach based on remanufacturing proven weapons when necessary offers significant advantages.

Ray E. Kidder

One of the most concrete — and now controversial — results of the US government's decision to end the testing of nuclear weapons was the subsequent decision by Congress¹ to require the Department of Energy (DOE) to establish a 'stewardship' programme, with the goal of preserving US competence in the field of nuclear weapons in the absence of nuclear tests. In response, the department has drawn up proposals for its Stockpile Stewardship and Management Program², which is now coming up for approval by Congress. This programme, which aims both to maintain "the core intellectual and technical competencies of the United States in nuclear weapons" and to ensure the continuing safety and reliability of the US nuclear weapons stockpile, has at its core the development of new experimental and computational capabilities, to provide a better understanding of the physics of nuclear explosions, and hence better predictions of the behaviour of weapons in all conceivable circumstances.

I will argue here that this programme, which is expected to cost \$4 billion per year for at least the next ten years, is misguided in a number of ways. First, it involves unnecessary changes in warhead material and designs, which could lead to reduced reliability and safety of the stockpile. Second, it underestimates the difficulty of maintaining competence in nuclear weapons design during a long-term comprehensive test ban. Finally, it calls for unnecessary new experimental and computer facilities which will not only be costly, but will also undermine arms control efforts by provoking doubts about their real purpose.

Reliability and safety

Nuclear weapons in the US stockpile are currently both safe and reliable. But metals corrode, and organic materials such as plastics, adhesives and high-explosives deteriorate with age. Such ageing effects degrade a warhead's reliability, but not its safety. The sensitivity to impact or fire of the high-explosives used in nuclear warheads, for example, does not significantly increase with age. In fact, safety problems with nuclear warheads are generally inherent in the design of the warhead itself, not the result of ageing or other causes³. Safety problems would therefore not be expected to arise unless the design of the 'physics package' — the nuclear explosive part of the weapon — were to be modified.

The inevitable effects of ageing mean that

each nuclear weapon in the stockpile will eventually need to be replaced. The challenge is to minimize the risk of degrading their present safety and reliability in the process. Although in principle it should be possible to remanufacture weapons identical to those currently in the stockpile, in practice it is not always possible to obtain materials that are strictly identical to those used a decade or more ago.

Fortunately, not all parts of a nuclear weapon require nuclear explosions to test whether they are working properly. Many components, including the arming, fusing and firing system, power supplies, and the like, may be changed as long as they provide the correct environment for, and inputs to, the physics package within the originally specified tolerances. New components can be fully tested to ensure that they meet this criterion.

This leaves the question of the physics package. The DOE has suggested² that some avoidable changes in the design of the package are "desirable", even though the effect of these changes on the most critical phase of its nuclear performance, the primary 'boost' phase, cannot be tested. Instead, it will be necessary to rely on computer simulations of this complex and sensitive physical process.

Remember that it is not necessary to improve the safety and reliability of the existing stockpile; its reliability has been demonstrated in many nuclear tests (typically seven or more) of each weapon type. But the DOE argues⁴ that "remanufacturing alone would not permit stockpile improvements to address reliability or safety concerns." Moreover, the department states that "remanufacturing would not retain the required breadth and depth of nuclear weapon expertise and judgement that will be needed to address future concerns about the safety and reliability of an ageing stockpile."

In other words, the DOE expects that the required level of nuclear weapons expertise and judgement can be retained only if it continues to be exercised by being actively

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Many of the more than 6,000 parts contained in a B-61 nuclear bomb are shown here, in front of a fully assembled weapon (rear) and a partially disassembled one. All of these components, except for the 'physics package' (indicated by a red arrow), can be tested without nuclear explosions.

engaged in a continuing programme of warhead modification, presumably including modification of the physics package. I believe that this expectation is unjustified, for reasons given below, and that such a programme will lead to a loss of stockpile reliability in the long run. The DOE rejects remanufacturing "alone" as a means of maintaining the stockpile, but I will argue below that if a programme of remanufacture is preceded by one (and only one) opportunity to modify the physics package to permit successful remanufacture, then the long-term safety and reliability of the stockpile can be maintained.

Maintaining competence

In addition to ensuring the safety and reliability of the stockpile, the stewardship programme is supposed to maintain US competence in nuclear weapons. As the DOE leaves the expression "core intellectual and technical competencies" undefined, I shall assume that it refers to a level of competence comparable to that of nuclear weapons designers and engineers currently employed at the Livermore and Los Alamos national laboratories. I believe that the DOE has underestimated the difficulty of maintaining such competence in the absence of nuclear testing, and that this underestimate has resulted in a proposed stewardship programme that could lead to future problems with the safety and reliability of warheads.

First-rate scientists and engineers seek challenging, innovative work that can be developed, tested and published; they certainly prefer this to the work of maintaining a moribund nuclear stockpile whose details must remain secret. They also prefer a job that is 'going somewhere'. Such individuals can be given attractive jobs related to weapons physics and engineering; indeed, that seems to be part of the motivation for the inclusion of new experimental and computer facilities in the DOE's programme. But this is not the

same as working directly on the detailed design of nuclear weapons themselves.

It is therefore unrealistic to assume that a staff of first-rate nuclear weapons designers and engineers comparable to those now at Livermore and Los Alamos can be maintained for the many decades during which they may be needed. A decline in competence concerning nuclear weapons is virtually inevitable. A recent demographic study at Los Alamos⁵ has shown that this predictable erosion of the nuclear weapons skill base is already under way.

With less competent designers and engineers to implement it, a programme of continuing modification of the physics package — believed necessary by the DOE to maintain competence — would in fact pose an unnecessary risk to the safety and reliability of the stockpile.

New experimental facilities

Warhead modification is not the only means by which the DOE proposes to maintain intellectual and technical competence. The Stockpile Stewardship Program also calls for the development of three new experimental facilities, with more to follow in succeeding years. The largest and most costly (\$1.2 billion) of the near-term projects is the proposed National Ignition Facility⁶ (NIF) — a powerful 192-beam laser system that will produce extremely high-temperature and high-density states of matter, and may be able to ignite a small pellet of thermonuclear fuel (deuterium plus tritium).

NIF and a similar French facility, Le Projet Mégajoule, will be the leading laser facilities of the international effort in inertial-confinement fusion (ICF). This effort has the short-term goal of demonstrating that controlled thermonuclear micro-explosions can produce a modest energy gain, and a longer-term goal of exploring their possible application to the generation of commercial electric power. In my view, the primary justification for NIF stems from the possibility it offers of creating thermonuclear micro-explosions in the laboratory for the first time, from its role as a unique and versatile instrument for basic and applied research in high-energy-density physics, and from its leading role in the ICF effort.

The relevance of NIF to nuclear weapons science is that the states of matter produced, and the physical processes involved, are similar to those that govern the behaviour of nuclear weapons. As a result, computer programs used in ICF research have much in common with those used in nuclear weapons design. The more powerful of these are therefore classified, at least at the three US nuclear weapons laboratories.

It should not be assumed, however, that NIF is essential for the continued safety and reliability of the nuclear weapons stockpile. Its primary contribution to the Stockpile

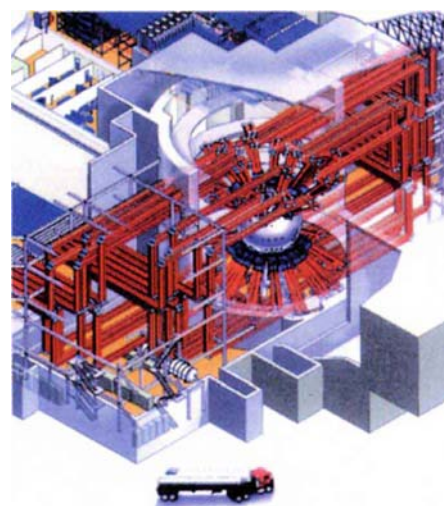
Stewardship Program is to recruit and maintain a staff of theorists and experimentalists actively engaged in the physics of shock waves, high-temperature hydrodynamics, radiative transfer and neutronics — the basic physics that underlies the design of nuclear weapons. But in contrast to thermonuclear micro-explosions, which begin with beam-driven fusion, nuclear weapon explosions begin with high-explosive-driven fission. Some of the physics is the same; but the details, 'wherein the devil lies', are quite different. It would therefore also be wrong to assume that NIF will be able to support for the long term a staff of weapons designers and engineers with detailed design competence comparable to that of those now working at the weapons design laboratories.

Some of the other experimental facilities included in the DOE's programme are genuinely needed to evaluate the condition of the stockpile and the performance of its component parts, but others are not. The first axis (\$81 million) of the Dual-Axis Radiographic Hydrodynamic Test facility (DARHT) at Los Alamos is needed to evaluate the dynamic performance of a nuclear weapon's primary implosion system. The Contained Firing Facility (\$50 million), another single-axis X-radiography facility, is needed for the same reason at Livermore.

But the need for the second axis of DARHT, and for the Atlas facility at Los Alamos (\$43 million) — a massive capacitor bank for creating electrically driven implosions which is intended to help evaluate problems with the secondary (fusion) part of the physics package — is questionable. And the need for the proposed future Advanced Hydrotest Facility (~\$400 million), Jupiter X-Ray Simulation Facility (\$240 million) and Next-generation Procyon, a high-explosive-driven pulsed-power system (\$70 million), has not been adequately demonstrated and probably cannot be.

Concerns about arms control

There is an important reason, besides cost, for the stewardship programme to include only those facilities that are genuinely needed to maintain the safety and reliability of the stockpile — namely, that new experimental facilities that are clearly not necessary for that limited purpose, and cannot be justified as a necessary component of a long-established non-weapons programme, could have an adverse effect on arms-control negotiations. Russia or China, for example, could conclude that the purpose of such facilities is to provide an enhanced capability that they themselves could not duplicate to design new, more sophisticated nuclear weapons, and object to the facilities on those grounds. This problem is exacerbated by the fact that the DOE does not rule out the possibility of using these enhanced capabilities to design 'new-design' nuclear weapons².



The National Ignition Facility, with its 192 laser beams, will play a leading role in fusion research, but is not essential for stockpile stewardship.

Another arms-control problem arises from the programme of underground tests that the DOE is planning as part of its stewardship activity. The United States is seeking a 'zero-yield' Comprehensive Test Ban Treaty⁷. A nuclear weapons test is defined to be 'zero-yield' if the fissile material of the weapon remains in a subcritical state during the test, so that no self-sustaining nuclear chain reaction can occur, and the amount of nuclear energy released is negligible. Chain reactions and limited neutron multiplication can be sustained in the presence of an external or internal source of neutrons, but will die out when the source is removed. Zero-yield tests can be used to assess some aspects of warhead safety and reliability, to investigate material properties, and to help validate computer programs describing neutron transport.

Nuclear tests that are truly zero-yield need not be conducted underground, as they can be carried out safely in above-ground containment facilities of modest cost and size. The justification for doing them underground at the Nevada Test Site is generally the convenience and simplicity of waste disposal, as the hazardous waste created by the test is sealed in place underground. There is also the small — but unacceptable — risk that the zero-yield limit could be exceeded in tests of nuclear detonation safety.

The underground tests currently being considered by DOE would be conducted at a depth of 300 metres. The problem is that this is sufficient to contain nuclear explosions with yields large enough to be of considerable value in developing new types of nuclear weapon. Although the United States has no intention of doing this, such tests would therefore set a precedent for conducting nuclear tests deep underground in a manner that a test-ban treaty violator would find ideally suited to his purpose. For this reason, I believe that zero-yield tests should be

conducted in above-ground or near-surface facilities, using appropriate test procedures to ensure sub-criticality. (See ref. 8 for additional discussion leading to this conclusion.)

Computer simulation

In addition to new experimental facilities, the DOE's programme provides for enhanced computational capabilities, to "fill the void... left by the termination of nuclear testing"⁴. Specifically, the Accelerated Strategic Computing Initiative plans to provide each of the three weapons laboratories with a parallel-processor supercomputer, at a total cost of over \$250 million. Two-dimensional computer simulations have long been used to design weapons and predict their nominal explosive yield. Three-dimensional simulations, which require significantly greater computer power, are used to evaluate a weapon's one-point detonation safety — that is, the yield that could result from an accident in which the weapon's high-explosive were detonated at any single point. One-point safety has been tested and verified in the past by nuclear tests of very low yield, and can also be tested in zero-yield tests. The purpose of three-dimensional simulations is to reduce the required number of tests by predicting the point or points of detonation that are likely to produce the largest yield. They are useful, but not essential.

For the past twenty years, the two weapons design laboratories, Livermore and Los Alamos, have been able to predict the yield of nuclear tests with remarkable accuracy (the specific value is classified), and to measure those yields by radiochemical means with an accuracy close to 5 per cent. (This figure is based on a statistical analysis of 170 nuclear tests conducted between 1975 and 1986, a period during which prediction accuracy remained substantially unchanged⁹.) Although such an accuracy would not apply to early tests of new weapon concepts (which occasionally did not work at all), it does apply to the well tested and certified weapons in the present stockpile. Thus, the weapon designer's skill, computer input data, computers and two-dimensional computer programs that have existed for many years, together with experimental tests of one-point safety, have sufficed to provide a safe and reliable stockpile of nuclear weapons.

Remanufacture: a safe approach

As discussed above, the reliability and safety of the existing stockpile has been demonstrated in many tests of each nuclear weapon type. Notwithstanding this proven reliability and safety, and the problem of declining competence, the DOE believes that a continuing programme of warhead modification would be necessary to address reliability and safety concerns, and to maintain competence. But a programme of repeated warhead modifications will not help to maintain

competence; instead, such a programme would require competence that will not be available some decades from now.

An alternative approach that would minimize the effect of declining competence would be to replace the physics package of a warhead by a modified version only once, and then to replace it with a remanufactured copy when replacement eventually becomes necessary. The one-time modification of the physics package is intended to make the changes needed for successful remanufacture, and to improve its reliability and safety if this can be certified without a nuclear test.

Such changes should be made soon, while the designers and engineers who developed the warheads now in the stockpile are still available to certify them. Components outside the physics package can be modified or replaced at any time, provided that the environment and inputs to the physics package itself remain unchanged.

The US nuclear weapons stockpile could be maintained certifiably reliable and safe for decades (or even centuries) by continuing the assiduous and perceptive surveillance that has been practised for many years. Warheads would be removed from the stockpile when such surveillance indicates this to be advisable or necessary. They would be replaced by new warheads that have been remanufactured within the tolerances and specifications of their predecessors.

Such a programme would preserve to the greatest extent possible the design of weapons whose nuclear explosive performance has been fully tested, and which are known to work. It would limit future modification of the stockpile by less experienced and competent hands, which could reduce or destroy its reliability. And it could not reasonably be interpreted as an attempt to further develop and produce novel weapons.

The DOE and the nuclear weapons design laboratories have long argued that remanufactured weapons would not be reliable. But this is plainly contradicted by the extensive record of US nuclear weapons tests. Since 1972, there have been 17 "stockpile confidence tests" — nuclear tests of warheads that were withdrawn from the stockpile and subjected to simulated stockpile-to-target environmental conditions before being tested.

The warheads used in these tests were aged production-line units, in one case nearly thirty years old. When their performance was compared with that of the original production units on which their design was based, it was found that in all but one of the warheads tested, the difference in age and means of fabrication had little or no effect. In that exceptional case, a militarily insignificant reduction in yield was observed that was readily corrected, with no need to modify the nuclear package¹⁰.

Neither these results, nor the accuracy of

yield-prediction described above, would have been possible if the performance of US nuclear warheads had not been comfortably tolerant of the small variations in materials and manufacture that accompany any practical production process. Indeed, it was the robust character of nuclear weapon designs that led nuclear weapons experts Hans Bethe, Norris Bradbury, Richard Garwin, Carson Mark, Andrei Sakharov and Herbert York, among others, to the conclusion that nuclear explosive tests were not required to maintain stockpile reliability¹⁰.

The DOE and other opponents of this approach point out that some materials used in nuclear warheads, such as plastics and adhesives, are no longer available, and claim that they cannot reasonably be made available. In such cases, the best one can do is to use replacement materials certified to perform the same function without degrading warhead performance.

But this should be done now, while the designers and engineers who developed these weapons are still available to certify the replacement materials. Eight years have already elapsed since the Congress called on the Secretary of Energy to "assure that the specific materials, components, processes, and personnel needed for the remanufacture of existing nuclear weapons or the substitution of alternative nuclear warheads are available to support such remanufacture or substitution if such action become necessary in order to satisfy reliability and safety requirements under a low-threshold or comprehensive test ban agreement"¹¹. The DOE should provide this assurance without further delay. □

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From the Labrador Sea to global change

Bob Dickson

An understanding is emerging of the powerful changes that have occurred in convection in the North Atlantic. That understanding provides pointers not only to the regional influence of such changes, but how they may bear on global shifts in climate.

Once upon a time, the intermediate layers of the ocean must have seemed a rather dull place for front-line science. Subtracting one hydrographic 'snapshot' from another, often separated by long intervals of time, it would have been clear enough that change was taking place. But such variability as was found must have appeared to be merely the slow-paced, low-amplitude and rather unsurprising reflection of events in the so-called 'active layer' of the upper ocean.

Not any more. As our hydrographic records have lengthened to decades, the changes revealed at intermediate depths have provided physical oceanographers with one riveting surprise after another; and in the process of assigning cause and effect, we have also begun to realize that these changes may have a regional, and even a global, significance. In the North Atlantic, the main cockpits of deep hydrographic change have been the localized centres of convective activity in the Greenland, Labrador and Sargasso Seas, where the winter formation and renewal of vertically homogeneous 'mode waters' provides a mechanism for carrying the signal of climatic change to intermediate and greater depths, and from there — by horizontal spreading — throughout the ocean basin.

On page 675 of this issue¹, Sy *et al.* report the latest in a string of unexpected developments from the convective centre of the Labrador Sea. Since the late 1960s, when convection there was at its weakest and shallowest (down to 1,000 m; ref. 2), we have witnessed a remarkably sustained re-intensification and deepening of convective activity. From 1966 to 1992, the overall freshening of Labrador Sea Water (LSW) has been equivalent to mixing-in an extra 6 m of fresh water at the sea surface; its cooling has been equivalent to a continuous loss over the 26 years of 8 W m^{-2} ; and by 1993–95, the depth of convective overturn had so exceeded its normal limits (2,000 m or so) that, for the first time in our experience, it began to excavate the top of the colder but saltier sublayer of North Atlantic Deep Water (NADW) at depths to 2,300 m (ref. 3).

It is as a result of this change that the density of the thick LSW layer underwent an

abrupt increase between 1990 and 1994–95, and it is this anomalously cold, fresh and dense new vintage of LSW that Sy *et al.*¹ use, together with its chlorofluorocarbon signature, to provide modern estimates of trans-ocean spreading rates at LSW depths. As we might almost have predicted by now, the results were quite unexpected, with spreading rates three or four times faster than previous estimates^{4,5}.

So much for the 'local' changes within the domain of Labrador Sea Water. As these events were working themselves through, a sideways glance at the other two convective cells would have revealed that they too were undergoing unprecedented changes, in phase but in the opposite sense to those in the Labrador Sea (see Fig. 1). Thus, as Labrador Sea convection ground slowly to a halt in the 1960s, the production and density of '18° Water' (the mode water of the Sargasso Sea) reached a postwar maximum⁶. And as the LSW layer cooled, freshened and thickened from the late 1960s to the 1990s, and its convection reached to new depths, so the deep waters of the Greenland Sea became warmer, saltier and thinner, and its depth of convective overturn dwindled from over 3,500 m in 1971 to under 1,000 m in the 1990s⁷. We now believe that we have an explanation for this pan-Atlantic coordination of extreme convective behaviour — simply that all three cells underlie the recurrent atmospheric circulation mode known as the North Atlantic Oscillation (NAO), and reflect a long-term alternation in the ocean's response as the NAO changed from its 'low-index' extreme state in the 1960s to its record 'high-index' state in the 1990s⁸.

The potential importance of these convective changes devolves from this link with the NAO. It is still very much 'potential' at present, relying on several convergent lines of thought for which the evidence is fairly new or unproven.

First, we have the observation that there is decadal 'system' to the temperature evolution of the upper ocean, with large, organized sea surface temperature (SST) anomalies circulating around the Atlantic gyres coincident with decadal changes in the NAO (ref. 9). We now have good evidence that these recurrent surface features track the

movement of deep-seated anomalies formed by winter convection and mode-water formation as they propagate cyclonically along a so-called 'warm water transformation pipeline' (ref. 10; M. S. McCartney, R. G. Curry and H. F. Bezdek, personal communication), which runs from an origin in the western subtropical gyre to its end-point in the Labrador Sea. Put simply, the mode waters and surface heat anomalies are linked and move.

Following up this point, a second line of enquiry tackles the question of whether these changes in SST and mode-water formation are merely the response of a passive ocean to a decadal evolving atmosphere, or whether (and how) the ocean might feed back to encourage long-period change in the NAO

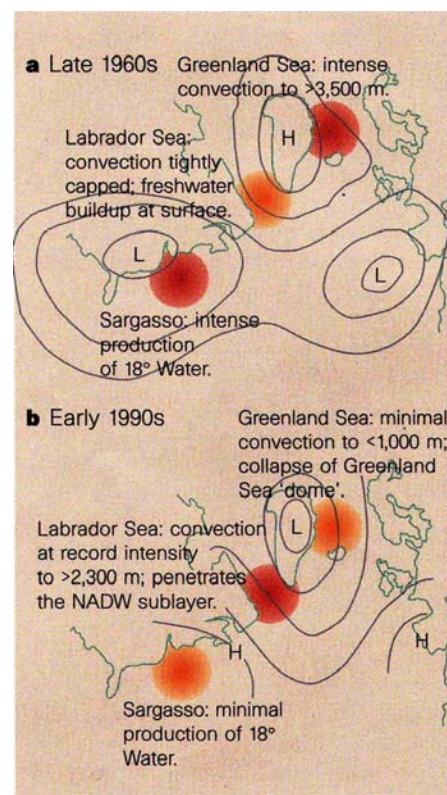


Figure 1 Depiction of the changes in the distribution of winter convective activity in the North Atlantic during contrasting extreme states of the North Atlantic Oscillation (NAO). The main convective centres are in the Greenland, Labrador and Sargasso Seas. **a** Represents the 'low-index' NAO conditions of the late 1960s; **b**, the 'high-index' state of the early 1990s. A representative mean pressure-anomaly field is indicated for each case. 'Mode waters' are formed in winter at each of the three sites, are vertically homogeneous, and through horizontal spreading provide a mechanism for carrying the signal of climate change throughout the North Atlantic basin. '18° Water' is the mode water of the Sargasso Sea. NADW is North Atlantic Deep Water. The Greenland Sea 'dome' is the place where a cyclonic basin circulation brings dense water closest to the surface, promoting convective instability.

between its 'low-index' and 'high-index' extremes. The slowness and persistence of that atmospheric evolution¹¹ suggests that the thermal inertia of the ocean is contributing 'memory' to the process; as if in confirmation, our longest instrumental and proxy measures of the NAO index show that its spectrum has actually reddened over the past 100 years or so¹²⁻¹⁴, with an ingrowth of spectral energy at periods of a few years to decades. Very recently, a range of new model runs suggests that the system may indeed be coupled (ref. 15; A. Timmerman, M. Latif, R. Voss and A. Grotzner, personal communication).

Making the link, finally, from the regional to the global level, we have evidence from multivariate linear regression analysis¹⁶ that the NAO and El Niño Southern Oscillation (ENSO) between them have accounted for much (44 per cent) of the variance in Northern Hemisphere extratropical (20°N–90°N) winter temperatures since 1935, with the NAO contributing by far the larger share (31 per cent).

Seen in this light, the coolings, freshenings, deepenings and spreadings of Labrador Sea Water no longer seem parochial. Far from being a small deal in the northwest Atlantic, these changes may well be playing the major role in the largest deal of all — protracting, repeating or in some way structuring the short-term behaviour of the NAO into the long, slow shifts of global change. As the Scientific Steering Group of the Climate Variability (CLIVAR) Program meets in Toronto and Washington to draw up its initial implementation plan (March and April 1997), the oceanographic component will certainly include our best ideas as to how to encompass and quantify change in the mode waters of the North Atlantic. But if our recent experiences are anything to go by, they had better build in some redundancy. □

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Gamma-ray astronomy

The first visible burst

Bohdan Paczyński and Ralph Wijers

Only a few things in astrophysics are as mysterious as γ -ray bursts — one might add to their company dark matter and the energy density of the vacuum (the cosmological constant). There is still debate over whether these rapid, one-off flashes of high-energy photons come from the neighbourhood of our Galaxy or from far greater, cosmological distances. But now the first optical counterpart to a burst is reported on page 686 of this issue by Van Paradijs et al.¹. At the same location there may be a fuzzy object, so faint that it stretches even the capabilities of our most powerful telescopes. If it is indeed a distant galaxy, the distance scale is almost certainly cosmological, and γ -ray bursts are confirmed as the brightest objects in the Universe.

Bursts were first discovered in 1973, by the Vela spy satellites², and they are now routinely registered at a rate of about one a day by the BATSE (Burst and Transient Source Experiment) instrument on board the Compton Gamma Ray Observatory³. Most of their energy is in γ -rays, hence the name. While bursting they dominate the sky — if that much energy was released in the optical domain, the brightest events would outshine any star for a few seconds. Bursts appear randomly in time at any location in the sky, and they are extremely diverse: the shortest yet seen lasted only 5 milliseconds, the longest a few hours, though most of them last between 1 and 100 seconds⁴. Some have rapid variability down to millisecond time-scales, some appear to be made of many independent components, and others vary smoothly, with a relatively rapid rise followed by a gradual decline.

There is no shortage of theories to explain

bursts — in fact, there are hundreds of them. During the two decades between 1973 and 1992, it was generally assumed that the bursts came from old neutron stars, at distances of about 100 parsecs from us, but there was no consensus about the mechanism. Comets falling onto neutron stars, star-quakes, nuclear explosions, magnetic flares and many other energy sources were suggested.

The situation changed dramatically in 1992, with the announcement of results³ from BATSE. This was the first experiment able to measure burst positions directly, and it is very sensitive. All bursts, strong and weak, were found to be distributed randomly over the sky, with no apparent bias towards the Galactic plane or centre. Also, there were relatively few weak bursts, indicating that beyond some distance their number density declines. In other words, the BATSE data demonstrated that we are located at the centre of a spherically symmetrical distribution of bursts. That was a shock to the astronomical community. The observed distribution clearly excluded the previously favoured Galactic disk origin, because the weak and presumably distant sources were not concentrated in the Galactic plane.

Today, the distance scale is still an issue. Judging from the number of papers published on the subject, a clear majority of investigators place γ -ray bursts at cosmological distances, a few gigaparsecs from us. A vocal minority puts them in the extended corona of our Galaxy, at about 100 kiloparsecs. There is no support for the distance of 100 parsecs, which had been considered firmly established before 1992. Now we wonder, is the distance only 1,000 times larger, or is it 30,000,000 times larger^{5,6}?

No matter which number we choose, all pre-BATSE models are firmly excluded; a small disturbance on the surface of a neutron star just wouldn't produce enough energy. Many of the new theories for cosmological-distance bursts involve instead the collision of neutron stars, and an ensuing relativistic fireball. Other models involve active galactic nuclei and their surroundings. But we have no direct evidence to support any of these theories. The strength of the argument for the cosmological-distance scale has nothing to do with physical models of the bursts; it rests entirely on their observed distribution properties.

The most serious difficulty in understanding γ -ray bursts is the absence of emission in any other part of the electromagnetic spectrum. But that is now changing, following the launch of the Italian–Dutch BeppoSAX satellite⁷ (Fig. 1). Its wide-field cameras can pin-

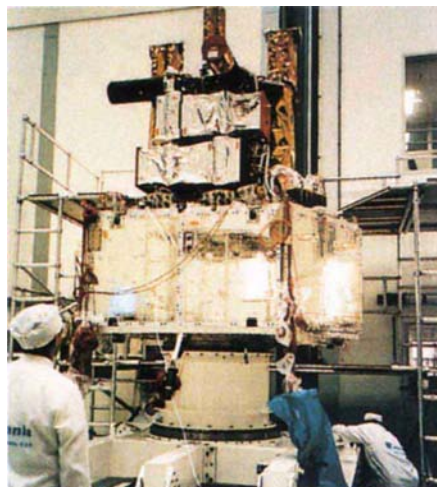


Figure 1 The BeppoSAX instruments. BeppoSAX can provide rapid and accurate burst positions with its wide-field cameras (silver-coloured).

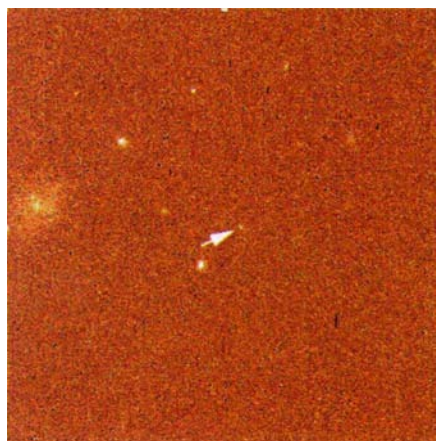


Figure 2 Burst afterglow. The optical counterpart to GRB970228 is in the centre; the bright object to the left and below is a cool star in our own galaxy, and there is a faint distant galaxy towards the upper right.

point bursts in 1–3 hours, and follow up with its X-ray telescopes. In the past, BATSE provided instant but inaccurate positions⁴, whereas the network of small γ -ray detectors on board interplanetary probes could measure very accurate positions by triangulation, but with a long delay⁴. In both cases the follow-up observations saw nothing.

The burst GRB970228 was reported⁸ by the BeppoSAX team on 2 March 1997, and four days later they reported an X-ray transient within the burst's position error box⁹. Van Paradijs and colleagues¹ describe how an optical transient was detected^{10,11} on 1 March, with an apparent magnitude of about 21, fading to fainter than about 23rd magnitude on 8 March. The Hubble Space Telescope detected the optical transient¹² at about 26th magnitude on 26 March, and slightly fainter¹³ on 7 April (Fig. 2). The 10-metre Keck Telescope and Hubble may also have found a very faint fuzz around the optical variable^{12–14}. But any of the definite galaxies that appear near the transient could be hosts — there will usually be a galaxy within such an area, so their presence doesn't favour an extragalactic origin.

It may take time before the counterpart problem is solved, as another similarly bright BeppoSAX burst, GRB970111, had no X-ray or optical transient source associated with it¹. X-ray luminosities are known to vary substantially between bursts with similar γ -ray brightness, and the variations in the optical band could be even greater. Fortunately, BeppoSAX has just reported¹⁵ yet another burst, GRB970402, from which it again saw an X-ray afterglow¹⁶. The first attempts to find an optical transient from this somewhat weaker burst were not successful¹⁷. But more robust statistics should be at hand before too long. If we are lucky — that is, if some obvious pattern becomes apparent — then we should have some understanding of the counterparts when a few more cases are dis-

covered, which may take another year or so.

What is the meaning of this exciting development? Many theoretical models predict X-ray and optical transients after Galactic or extragalactic γ -ray bursts. The very simplest model for the afterglow of a blast wave¹⁸ fits the X-ray and optical data from GRB970228 remarkably well. But theory is at an early stage, and hence flexible; and we should not forget that all of the pre-BATSE models turned out to be wrong.

It is unlikely that a definite theoretical understanding will be possible without new observations. We have yet to learn how common these transients are, how often there is a fuzzy patch near the burst location, what the spectra are, whether there are any spectral lines, and whether the redshift (if any) can be measured. BeppoSAX has provided the first breakthrough since BATSE. Now the critical issue is whether the earliest 10-arcminute positions can be made available to all interested observers on the 1–3-hour timescale on which they become known, to maximize

the chance of frequent and diverse follow-up observations. Timing is everything. □

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Palaeontology

Genesis of snakes in exodus from the sea

Nicholas C. Fraser

In the Garden of Eden, the serpent — portrayed complete with limbs by Michelangelo on the ceiling of the Sistine Chapel — was commanded to go on its belly and eat dust for the rest of its life (Fig. 1). But it seems that it took to the sea before it finally (more-or-less) settled down to an existence on land; at least, that's the view of Caldwell and Lee¹, who describe (on page 705 of this issue) a snake that has legs.

Searching the fossil record for clues as to the origin of snakes is a frustrating business. Snake skeletons are very delicate structures that are not readily fossilized. Most of the snake fossil-record comprises vertebrae, and "there is no fundamental difference between

the vertebral morphology of snakes and other modern Lepidosauria"². So it is little wonder that the search for the origin of snakes has proved to be a thorn in the side of vertebrate palaeontologists.

In looking for a plausible ancestor, we can certainly narrow the odds. Snakes (Ophidia) are clearly highly derived members of the order Squamata ('scaly reptiles'), which also includes the lizards (Lacertilia) and amphisbaenids (the worm-lizards). But within the Squamata, where is the safest bet? It seems unlikely that forms with well-developed limbs or a marine habit could be the ancestors of modern-day snakes. However, Caldwell and Lee lean towards just such a long shot. ►

IMAGE
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Figure 1 The fall of the serpent. In his famous scene, *The Fall of Man*, on the ceiling of the Sistine Chapel, Michelangelo depicted the serpent as having forelimbs.

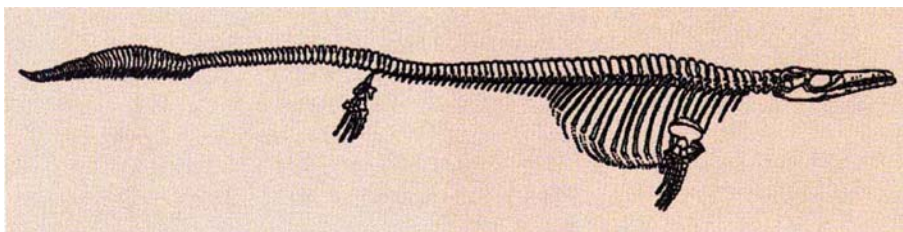


Figure 2 Caldwell and Lee¹ believe that the ancestors of modern serpents are a group of extinct marine lizards called the mosasauroids. These include the giant marine lizard *Plotosaurus* from the Upper Cretaceous, which was around ten metres long. (Adapted from ref. 7.)

The first step is to qualify what characterizes a snake. The popular image is that of a reptile completely devoid of limbs. But the absence of a character is not a valid criterion for assigning a group — in terms of evolutionary history, it is relatively easy to lose something. By contrast, acquisition of a particular character is not readily repeated, so the presence of a specific character is a more valid indicator of a close relationship. Moreover, one feature is rarely enough to draw the boundary between two sister groups. Snakes have a range of diagnostic characteristics, including the complete closure of the braincase wall, the lack of a sutural contact between the premaxilla and maxilla (jawbone), the dorsal contact of the exoccipitals above the foramen magnum, suspension of the quadrate from the supratemporal, and a mobile intra-mandibular joint.

Until now, snakes were believed to have evolved from a group of fossorial (burrowing) squamates — after all, the most primitive groups of snakes are largely burrowers, for example the Typhlopidae (blind snakes). Although loss of a character (in this case, legs) should not carry too much weight in phylogenetic analyses, the search for a snake ancestor has mainly concentrated on 'snake-like' squamates, with reduced limbs and long bodies. So, squamates that are, at least on the surface, completely limbless — notably the anguids (slow worms and glass snakes), dibamids and amphisbaenids — have been considered to be prime candidates.

If snakes were indeed derived from a burrowing, legless squamate, the concept of a snake with legs seems implausible. But even those squamates with no apparent external limbs usually have some remnants of the limbs or pelvic girdle. Furthermore, all living boas have traces of a pelvic girdle, and even limited vestiges of the hindlimb. So perhaps the prospect of a snake with legs is not as improbable as it might have at first appeared.

Enter *Pachyrhachis*, an unusual reptile that is known on the basis of two specimens from the mid-Cretaceous, which were discovered by George Haas 20 years ago in Israel. *Pachyrhachis* was indisputably marine rather than terrestrial, and the metre-long fossils have tiny, but well-formed, hindlimbs. Although Haas first described them as snakes, he later assigned

them to the varanoid lizards^{3,4}.

Caldwell and Lee¹ have now re-examined *Pachyrhachis*, and they have come up with some rather unexpected results, reviving an idea that was proposed over 100 years ago by the palaeontologist Edward Drinker Cope. The authors found that *Pachyrhachis* possessed most of the definitive characters of snakes, but they claim that it also shared certain traits with the mosasauroids, a group of extinct marine lizards that includes the giant mosasaurs of the Cretaceous seas (Fig. 2). One of the criteria that they believe link *Pachyrhachis* and the mosasauroids is the separate astragalus and calcaneum — these elements are fused in all other squamates that possess an ankle.

Although *Pachyrhachis* can now be regarded as the most primitive snake known, it is not the oldest — that honour still belongs to *Lapparentophis* from the Lower Cretaceous of North Africa⁵. This indicates that the origin of snakes probably extends back into the Jurassic. Whereas the giant mosasaurs were restricted to the Upper Cretaceous, the mosasauroids are a less exclusive group of marine lizards. The oldest is an aigialosaur from the Upper Juras-

sic, so the ages of these fossils are not inconsistent with a mosasauroid origin for the snakes. Nevertheless, arguments linking snakes with mosasauroids still face a stiff challenge. For example, the separate astragalus and calcaneum is not a particularly robust character, as these bones are also separate in many other lepidosaurs (although, admittedly, not the squamates).

It is still possible that the features shared by mosasauroids and *Pachyrhachis* are simply the result of convergence — sharing a similar aquatic lifestyle, rather than sharing a close phylogenetic relationship. Ultimately, irrespective of whether Caldwell and Lee's interpretations are correct, it will probably be hard to deny that, in the long run, they are searching in the right area. At the annual meeting of the Society of Vertebrate Paleontology in New York last October, Eitan Tchernov and colleagues⁶ discussed all the reptiles from the Ein Jabrud locality. They linked another (as yet unnamed) taxon with snakes and mosasauroids. Ein Jabrud is rich in marine vertebrates (including sharks with soft-part preservation), and it is probably critical to our understanding of snake origins. But it will take more discoveries before the dust finally settles on the debate. □

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Developmental neurobiology

Charting orientation territories

Rachel O. L. Wong

Our nervous system is wired up in a precise manner, to make sense of the vast amount of diverse information that it constantly receives. To understand how this intricate wiring is so carefully put together, much work has gone into devising ways to manipulate the normal sets of instructions that shape neuronal circuits during development. It is with this approach that Weliky and Katz¹ (page 680 of this issue) have brought us closer to understanding how electrical activity contributes to the wiring-up of a unique pattern of connections in the developing visual system. Their results provide us with a clearer view of the developmental relationship between the electrophysiological properties of a neuron, and those of its neighbours.

Vision is a process by which light information from the retina — for example, colour, form or motion — is conveyed along many

different channels, which are later recombined in a complicated, but systematic, fashion by the primary visual cortex. Despite the complexity of these connections, mapping of visual information to the brain is highly stereotypic; that is, neurons that are responsive to a particular visual stimulus are often grouped together into layers, stripes, columns or patches². So how do cells in the developing visual system acquire their different response properties, and how does each property become distributed in such stereotypic fashion among the population of cells in the brain?

In the 1960s, Hubel and Wiesel³ showed that visual cortical neurons are most responsive to light and dark edges of a particular orientation in space. For example, whereas a moving edge evokes a strong electrical response in a cortical cell when it is presented horizontally in the visual field, the same stimu-

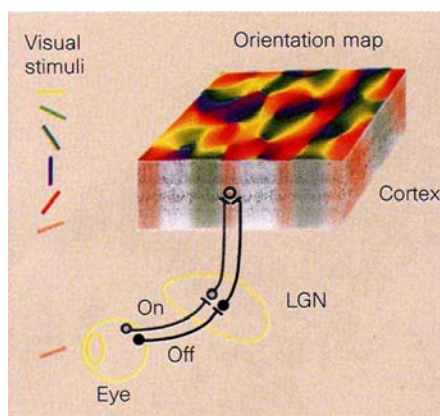


Figure 1 Cells in the primary visual cortex are sensitive to particular orientations of the visual stimulus: for example, cells that respond well to stimuli that are presented vertically (blue) do not respond to horizontally orientated stimuli (yellow). The orientational bias of cortical cells is built on the inputs from on- and off-type channels from the retina and the relay centre, the dorsal lateral geniculate nucleus (LGN). Within the cortex, cells with a similar orientational bias are distributed in a patchy but systematic configuration, forming orientation maps. Weliky and Katz¹ now show that the mechanisms that form these maps are not affected by electrical messages that come from the retina during development (adapted from a figure by L. C. Katz).

lus is ineffective when it is vertically orientated. Considerable effort, both experimental¹⁴⁻⁷ and theoretical⁸, has gone into trying to work out how cells in the developing and adult visual cortex acquire their 'orientation preference'. Although the wiring of connections that underlie this property remains elusive, orientational bias seems to require inputs from on-centre and off-centre-type retinal neurons². On-centre and off-centre responses are evoked when retinal projection-neurons are stimulated, or suppressed, respectively, by light onset. In mammals, the two responses are communicated as separate channels of information from the retina to a relay centre in the brain (the dorsal lateral geniculate nucleus). They are then combined at the level of the cortex, to shape the electrical responses of cortical neurons (Fig. 1).

Hubel and Wiesel¹ not only showed the orientational bias of cortical cells in response to light; they also found that cells with the same orientation preference are organized into patches that show a repeating pattern across the surface, and within the depth, of the cortex. This pattern gives rise to what is known as the 'orientation map' (Fig. 1), which is easily viewed using optical recording techniques. These provide images of the spatial distribution of activity in response to stimulation, at the cortical surface of the living animal⁵⁻⁷. In studying how these spatial patterns are set up during development, Weliky and Katz¹ have asked whether the mechanisms that shape the

orientation preference of individual cortical cells also dictate their spatial organization.

One such mechanism may be that of electrical activity, which is crucial for establishing specific patterns of connections in other parts of the visual system⁹. Visual experience is thought to set up the orientational bias of cortical cells but, although activity is important, visual experience itself may not be vital^{4,6,7}. However, until now it was unclear whether activity *per se*, or specific temporal patterns of activity, plays the instructive role.

Theoretical studies indicated that distinct temporal patterns of activity influence the final organization of neuronal connections during development⁸. To test this, the aim has been to manipulate the pattern of endogenous activity that is delivered to the cortex during the period when visual connections are formed. One way to do this is to artificially stimulate the optic nerve to produce patterns of synchronous or asynchronous activity that are not normally present¹⁰. Now, with much perseverance and technical finesse, Weliky and Katz¹ have chronically stimulated the optic nerve, and artificially synchronized the activity of on and off inputs to the visual cortex during the period of development when orientation tuning and map formation takes place.

The results obtained by Weliky and Katz

elegantly show that although the orientation tuning of cortical cells is weakened by artificial synchronization, orientation maps still develop. Kim and Bonhoeffer⁶ have already shown that orientation maps are resilient to changes in activity patterns after their initial formation, but Weliky and Katz have extended these interventions to the developmental period before orientation maps appear. They have shown that, even then, specific patterns of activity do not seem to be crucial for this unique pattern of connectivity to emerge.

What do these results really tell us about how the brain organizes its connectivity patterns during development? The important message from the new work is that the mechanisms that confer a particular physiological property on a cortical neuron do not necessarily ensure that its immediate neighbours are treated similarly. Stereotypic arrangements such as orientation maps may be intrinsic to the cortex (perhaps they are dependent on local connectivity patterns within the cortex^{8,9}), and they are undisturbed by the electrical messages that are conveyed from the retina during development. This contrasts with the formation of ocular dominance columns in the cortex, which depends highly on input activity⁹. ►

Animal locomotion

The cheetah's time has come

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Everyone knows that the cheetah (*Acinonyx jubatus*), shown here in full flight, is the fastest land animal. So a report by N. C. C. Sharp (*J. Zool.* 241, 493-494; 1997) claiming to provide the first accurate measurements of the cheetah's running speed comes as something of a surprise. Moreover, the timing was done as long ago as 1965.

Apparently, for many years after Sharp made the measurements, he was unaware of the inaccuracy of previous studies. The widely quoted report of a cheetah running at 71 m.p.h. (114 km h⁻¹) was discredited some time ago — not only was the distance over which the speed was measured found to be 65 not 80 yards as first thought, but the timing was inaccurate and the arithmetic faulty. Other calculations have been made from films, using the animal's body length to estimate the distances involved. These yielded a disappointingly low estimate of 56 m.p.h. (90 km h⁻¹),

suggesting that the cheetah might be outspurred by a Thomson's gazelle — though timings of the speed of the gazelle are themselves subject to doubt.

The speed for the cheetah now reported was assessed very simply. Sharp, then an athletics coach, who says he was "well used to hand timing", measured a course of 220 yd (201.2 m), marked at either end by posts and a thin tape. The cheetah was held 18 m from the posts, to allow a running start, and when its attention was caught by a meat lure held from the back of a Landrover, it was released. The cheetah chased the Landrover over the course, and was timed with two calibrated stopwatches.

Three separate runs yielded almost identical speeds — the fastest was a rather impressive 65 m.p.h. (105 km h⁻¹), still much faster than racehorses at 69 km h⁻¹, or greyhounds at 58 km h⁻¹.

Helen Phillips

The apparent diversity in how cortical territories that process similar forms of information are charted during development encourages further investigation — both into how this process occurs, and why such territories need to be parcelled out in the first place. □

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Ecology

Bog standards in Minnesota

Peter D. Moore

The idea of a raised bog may seem something of a contradiction in terms — most wetland habitats are low-lying and receive drainage water from a surrounding catchment. But the raised, or domed, bog is a very widespread, if declining, type of mire in the temperate zone of the Northern Hemisphere, especially in the more oceanic regions (Fig. 1). The debate about how a wetland can maintain its surface several metres above the groundwater table and yet avoid desiccation has simmered for a century. Do these domed masses of peat pull up water from below by capillarity? Or do they impede the downward movement of rain water as a consequence of the impermeable nature of compacted peat? Or are they dependent on hydrostatic pressure from below, as is suggested by research by Glaser, Siegel, Romanowicz and Shen reported in the current issue of *Journal of Ecology*?

Raised bogs are dome-shaped structures, often several kilometres in diameter and achieving peat depths in excess of 10 m. They develop in a variety of ways, often as a result of the infilling of lake sites by plant successions that run from swamp forests to communities dominated by bog moss (*Sphagnum* spp.), which is usually the main peat-forming vegetation of the rising peat dome. Such bogs may also arise within estuarine sites or over alluvial swamps and fens, as in the fenlands of eastern Britain.

The exploitation of the peat deposits as a source of horticultural soil conditioners, or of energy, has led to the loss of many raised bogs, especially in highly populated regions of the temperate zone such as Britain and Germany. Some of the areas where peat has been removed most effectively are also zones of lower precipitation, as in the case of south-east England, so most studies of raised bogs have been conducted in the high-precipitation regions where these mires are most frequent. The conditions necessary for the formation of raised bogs in relatively drier areas are consequently poorly understood.

The accumulation of large volumes of peat during the formation of a raised bog clearly demands waterlogged conditions in which microbial activity is impeded and decomposition fails to account for the totality of litter formation. So the problem arises as to how a raised bog can maintain surface wetness. The remarkable water-holding capacity of *Sphagnum* mosses (up to 20 times

num spp.), which is usually the main peat-forming vegetation of the rising peat dome. Such bogs may also arise within estuarine sites or over alluvial swamps and fens, as in the fenlands of eastern Britain.



Figure 1 High mire — a raised bog, Cors Fochno, west Wales.



100 YEARS AGO

When mine host in the ideal country inn, which all of us seek but none of us find, brings up a bottle of crusted wine covered with cobwebs and dust, this outward and visible sign is taken as convincing evidence of age. We grieve to have to record that the trust may now be misplaced. A *Bulletin* (No. 7) of the Division of Entomology of the U.S. Department of Agriculture says that in France and Pennsylvania an industry has recently sprung up, which consists of the farming of spiders for the purpose of stocking wine cellars, and thus securing almost immediate coating of cobwebs to new wine-bottles, giving them the appearance of great age. ... This application of entomology to industry is one which will not be highly commended.

From *Nature* 15 April 1897.

50 YEARS AGO

An Ecstasy. By Marie Carmichael Stopes Pp. iv + 19. (London: Alexander Moring, Ltd., 1946.) 5s.

Of the lyrical quality of this poem, as indeed of all Dr. Stopes' verse, there is no question:

"Brown rocks have sucked in all the sun's warm rays

To over brimming, till they fill the air
To fine enraptured whirlpuffs gliding where
They quiver up the bank of sheltered bays",

... But the author's enraptured journey tends to veil her powers of discretion. Imagination is not necessarily art.

"Soon my salt tanged palms
Vie with intoxicating scent brewed balms,
To yield sweet odours to the sun
drenched sky.

Short golden hairs, minutest flagstuffs rear
Each on its tiny mound, but soon they
cling

Content to nestle close like folded wing." Avoiding this misguided sensuousness, Dr. Stopes would be a poet of quality, and a stronghold for readers who deplore the absence of buoyancy in modern poetry. — Margaret Howard.

From *Nature* 19 April 1947.

Many more extracts like these can be found in *A Bedside Nature: Genius and Eccentricity in Science, 1869–1953*, a 266-page book edited by Walter Gratzer. Contact David Plant (e-mail: subscriptions@nature.com).

their dry weight) is undoubtedly a contributory factor, and led to raised bogs being described by the late Sir Harry Godwin² as "huge flat sponges". It is the nature of a sponge to hold water against gravity by the matric forces of capillarity, and this view of the hydrology of raised bogs was generally accepted until relatively recently³ — despite the fact that this notional model of the system could not account in physical terms for the high water table found within the peat domes.

In 1982 Ingram⁴ developed a model of raised-bog hydrology based upon the concept of the two-layered bog structure that had been put forward by Ivanov in Russia⁵. In this model a thin layer of actively developing peat (the acrotelm) overlies the bulk of the peat mass (the catotelm) which is more compacted and is permanently saturated with water. The hydraulic conductivity of the acrotelm is high (that is, water moves easily

through it), but the hydraulic conductivity of the catotelm is low, so the deeper layers of peat are effectively impermeable to either the lateral or vertical movement of water. In Ingram's model, the water supply of the raised-bog surface comes entirely from rainfall; and because this water cannot drain vertically through the peat dome, it moves laterally through the acrotelm, and the water which does not evaporate is ultimately shed at the dome edges. Rather than a simple sponge, the raised bog is like a hemisphere of solid rubber coated with a thin layer of sponge and is fed by water falling from above.

The dependence of raised bogs upon rainfall for their water supply (ombrotrophic mires) is the essential feature that differentiates this type of mire from fens (flow-fed, rheotrophic mires). It is interesting to find, therefore, that raised bogs can develop even in continental areas subject to relatively low precipitation, as in the northwest of Minnesota

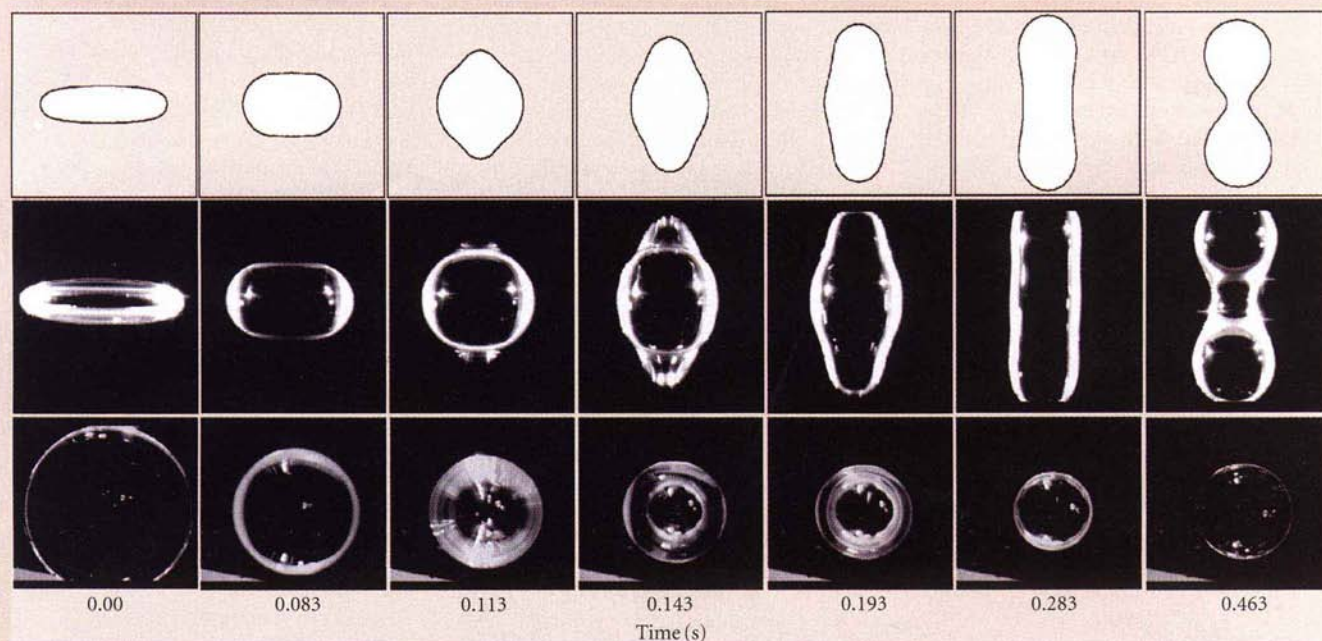
where annual precipitation is only 55–64 cm.

It is here that Glaser and his colleagues¹ have re-examined hydrological models of peat development. They studied 127 of the abundant domed mires in a wetland region of flow-fed fens, and conducted surveys of hydraulic head gradients through wet and dry weather conditions. They also analysed the chemistry of pore water to determine the influence of groundwater. They discovered that raised bogs are situated over discharge regions for groundwater so that during dry periods there is an upward movement of water from the peat/mineral base into the dome. The effect of this is to maintain a higher water table within the raised mire system. In wet periods, however, there is a reverse movement as a consequence of the excess supply of water at the surface from rain.

These findings raise some important questions for wetland ecologists. Are raised bogs in more continental climates generally

Fluid dynamics

Long drops in free fall



There is something fascinating about things in free-fall. Remember watching the crew of Skylab chase wobbling globules of drink around with a straw? Scientifically worthier, but perhaps just as much fun, is a new study of superdeformed drops in zero gravity, performed on the Space Shuttle Columbia and reported last month (Apfel, R. E. *et al. Phys. Rev. Lett.* 78, 1912–1915; 1997).

A single water drop "the size of a ping-pong ball", is initially held in an extremely oblate shape by the sound

from four powerful loudspeakers. The speakers are turned off, and the blob oscillates freely. The first half-oscillation is shown here: within less than half a second, the pancake becomes a lemon, a sausage and finally a peanut.

Such extreme deformations would not be possible without the addition of some surfactant, or detergent, which reduces the surface tension of the drop and so prevents the narrow neck in the last image from pinching off, leaving two drops behind. Here, just

1.4×10^{-4} grams per millilitre of the chemical Triton X-100 is enough to substantially change the bulk flow properties of the fluid.

But the details of surfactant behaviour in this nonlinear, nonequilibrium regime aren't well understood. Fortunately, the approximate behaviour of the drop can be predicted by numerical calculations that assume a uniform surface tension; the upper row of outlines shows the result. Comparing the prediction with the real blob, Apfel *et al.*

found that the fluid's dynamic surface tension was higher than its measured static value. That may be because the surfactant doesn't have time to reach saturation levels on the whole surface during each phase of the oscillation.

As well as the obvious relevance to the behaviour of surfactant-bearing liquid drops, the authors speculate that their experiment may provide insights into exploding stars and hyperdeformed atomic nuclei. **Stephen Battersby**

dependent on upward movements of groundwater? Are they therefore periodically decoupled from climatic control? If this is so, then the use of bog stratigraphy as a climatic proxy⁶ in such regions must be brought into question. Is the hydraulic conductivity of the catotelm peats in these continental raised bogs higher than in those from oceanic regions? If so, what are the structural differences in these peats? And can drought bring groundwater mineral supply within the reach of the roots of surface vegetation? Such reversals of the normal successional development towards low nutrient status and acidity have been previously recorded from the Minnesota area⁷. We can no longer

assume that elevation automatically results in ombrotrophy and perhaps we need to redefine the term 'raised bog'.

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Cell biology

The importance of being unfolded

Kevin W. Plaxco and Michael Groff

Dogma has it that the biologically significant state of every protein is the unique and well-defined native structure. Considering the folding of a protein from an unfolded conformation to its native state (U→N), much research has been done on the 'N' form, as well as on what is represented by the arrow, yet surprisingly little attention has been paid to the unfolded state. Developments in nuclear magnetic resonance and computer modelling techniques have made it feasible to start filling the gap and to investigate exactly how unfolded and random the 'U' form is. And it now emerges that dogma may be wrong in depriving unfolded proteins of any biological importance.

The strongest case for biological activity in an unfolded protein is presented by Daughdrill *et al.*¹ in this month's issue of *Nature Structural Biology*. The protein in question — the transcriptional regulator FlgM from *Salmonella typhimurium* — controls the supply of building blocks for the synthesis of flagella (Fig. 1). Flagella are the external filaments that are spun around like a propeller when bacteria swim², and each flagellar filament is attached through a flexible hook to a basal body, which anchors it in the cell membrane. Flagellar filaments contain an estimated eight per cent of the total cell protein, and their construction is controlled by a tightly regulated hierarchy of more than 50 genes.

For some time, researchers were puzzled by the observation that mutations in any of the three-dozen genes that are required for building the basal body and hook would induce FlgM to suppress the synthesis of the proteins required to form the filament. Bacteria build flagella very much like one would build a tower without having a crane: they transport the building blocks through the hollow centre of the flagellum to the end of the growing filament. The mystery of the regulation by FlgM was solved by the remarkable

finding that it is exported through this same channel, as long as open hooks and incomplete flagella are available³. When all of the flagella are completed, the export channels are closed and FlgM starts to accumulate inside the cell, where it can regulate the expression of other genes by binding to the flagella-specific transcriptional activator, σ^{28} .

By studying this molecular-recognition event using multidimensional NMR, Daughdrill *et al.*¹ have now found that unliganded FlgM is largely unfolded under physiological conditions. Only on binding to σ^{28} does the carboxy-terminal half of FlgM attain a folded conformation, however, the first 40 amino acids remain unstruc-

tured in both the bound and free states.

The observation of an unstructured protein *in vitro* might have been dismissed as being due to an inability to recreate the cytoplasmic environment. But in this case, there is a plausible reason for FlgM to remain unfolded *in vivo*. The inner diameter of a bacterial flagellum is about 2.5 nm, so a globular protein with the molecular weight of FlgM — which could be as big as 3 nm in diameter when folded — might not fit through the export channel. In contrast, an unfolded polypeptide chain could be threaded through the channel more easily. So, the as-yet unknown export mechanism could be the evolutionary cause that precludes FlgM from being like other proteins and having a folded native state.

It is not clear how far these findings can be generalized, but certain other proteins show a similar behaviour, suggesting that FlgM is not the exception that proves the rule. The cell-adhesion proteins from various Gram-positive bacteria^{4,5} (known as MSCRAMMS, for 'microbial surface components recognizing adhesive matrix molecules') and the cyclin-dependent kinase inhibitor p21^{waf/cip1/sid1} (ref. 6) have been shown, both by far-ultraviolet circular dichroism and by NMR spectroscopy, to be unstructured under plausibly physiological conditions. Like FlgM, these proteins fold to form a tightly bound complex in the presence of ligand.

Another potential example is the SH3 domain of the *Drosophila* signal-transduction protein, Drk. NMR spectroscopy indicates that within a population of Drk molecules, a considerable proportion is unfolded under physiological conditions⁷. The rate of inter-

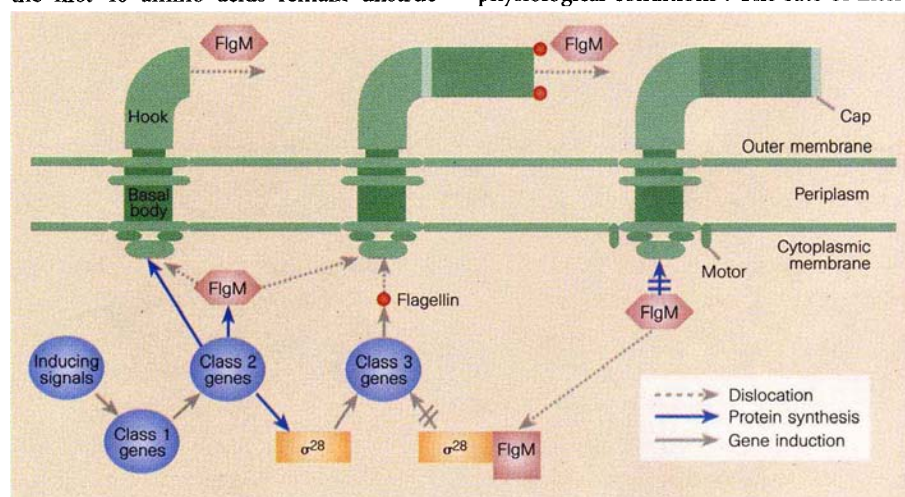


Figure 1 The morphogenesis of bacterial flagella is controlled by a tightly regulated set of more than 50 genes, organized in a hierarchical array of three classes. Class 1 genes are the primary targets of environmental signals. Their protein products induce the transcription of class 2 genes, which code for the basal body and hook proteins, the regulatory protein FlgM and the flagella-specific sigma factor, σ^{28} . The latter induces the transcription of class 3 genes, which code for the building blocks of the flagellar filament. Class 3 genes can be suppressed by mutations in any of the 35 class 2 genes because the class 2-encoded suppressor, FlgM, is exported from the cell through a channel in the basal body and hook structure³. If this export fails, FlgM accumulates in the cell and suppresses class 3 genes by binding to σ^{28} . Daughdrill *et al.*¹ have now shown that FlgM is unfolded in its active state, potentially because of the requirement to fit through the export channel.

conversion between the unfolded and the folded forms has also been monitored⁸: with a folding time-constant of around one second, unfolded protein molecules can presumably reorganize themselves to bind a target peptide rapidly enough that peptide binding and signal transduction are not impeded.

If the biological activity of unfolded proteins turns out to be a widespread phenomenon, how do these proteins escape degradation and aggregation in the cell? It is quite possible that unfolded yet active proteins can only occur on the cell surface, or in roles where they are found only at low concentrations in the cell — as with FlgM, which is either exported or bound to σ^{28} .

The awakened interest in the unfolded state corresponds with a 'new view' of protein folding⁹. This replaces the models of discrete folding pathways by multipathway 'energy landscapes' and defined intermediates by a distribution of partially folded conformations. Studies of unfolded proteins, both by NMR and by Monte Carlo simulations, have converged on the view that unfolded proteins sample backbone conformations that are remarkably similar to those found in fully folded proteins¹⁰. This presumably biases the conformational search that is undertaken by the polypeptide chain during folding, and is one of the mechanisms by which folding can avoid the astronomically long times that the Levinthal paradox predicts for a random search mechanism.

New evidence for the biological relevance

of unfolded states has also arisen from studies of pathogenic proteins. Unfolded conformations seem to be critically involved in the transformation from normal to pathogenic forms of the prion, and of proteins that are implicated in amyloidosis-related diseases such as Alzheimer's^{11,12}. We anticipate that future developments in this field, together with the insights gained from the 'new view' perspective, will lead to a more widespread appreciation of the idea that proteins are dynamic systems, sensitive to evolutionary modulation even in their unfolded conformations. □

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Climate change

A greener north

Inez Fung

For whatever reasons, the period since 1980 has been the warmest in the past 200 years. Every gardener can speculate about the fate of his garden in response to the warming, but the bigger question is whether the biosphere has responded in some larger way to the climate perturbation. Sustained long-term observations of photosynthesis are rare. Furthermore, the biosphere is notoriously heterogeneous. It is very difficult to extrapolate from field measurements at a few sites to behaviour over a large region.

On page 698 of this issue¹, Myneni *et al.* present satellite evidence that, on average, the biosphere between 45° N and 70° N has been enjoying increased photosynthesis between 1981 and 1991. The authors suggest that temperature increases in the spring time have also brought a longer growing season to the high latitudes. The evidence presented by Myneni *et al.* is the first direct observation of the biosphere that photosynthesis has increased on such a broad scale for such a long time. The satel-

lite observations are extremely provocative and, the authors argue, reveal specific areas where changes have occurred.

Remote sensing of photosynthetic activity is based on how plants use or dispose of solar radiation at different wavelengths. Green leaves absorb more than 85% of the incoming solar energy in the visible part of the spectrum and under 40% of the energy in the near-infrared: hence there is a large difference between the reflectivities at these wavelengths. By contrast, the reflectivities at these wavelengths are comparable for bare soils. A commonly used parameter for monitoring photosynthetic activity is the normalized difference vegetation index (NDVI): the difference between the reflectivities at the two wavelength bands normalized by the sum. A high NDVI is indicative of vigorous photosynthetic activity.

Satellite observations provide a global view of the land surface in days to weeks, depending on the days without clouds to obscure the view. The pioneering work of

Tucker and his co-workers^{2,3} has demonstrated the usefulness of the NDVI for deducing vegetation classes on a continental scale and for monitoring photosynthetic activity on a global scale. These studies employed satellite observations for a single year or a few years, and centred on the spatial and seasonal information contained in the NDVI distributions.

Looking for long-term changes in the satellite data is a challenge. The instruments were designed for weather applications in which the time scale of interest is days. The 11-year NDVI time series have been constructed from data from three radiometers on board three different polar-orbiting meteorological satellites. The performance of the radiometers diminishes with time, and at different rates for different wavelength channels. Furthermore there is the annoying interference by the intervening atmosphere. By selecting the maximum NDVI value over a period of 10 or 15 days, one hopes to have avoided clouds, which, being reflective in the visible spectrum, would yield a low NDVI. Also, because of the satellite viewing and solar geometries, there are differing amounts of atmosphere between the sensor and the surface; more importantly, the abundance of other atmospheric constituents (aerosols, for example) that interact with the radiation is not uniform in space or time. These must be removed before a clean signature of the surface can be obtained.

Myneni *et al.*¹ have taken a bold new step forward in extracting a secular trend from the NDVI information. They used two NDVI datasets, independently derived from the same raw satellite data. Each dataset includes a *post facto* correction for instrument calibration and atmospheric effects. As the authors point out, the correction is not complete, but they are confident that the NDVI trend at high latitudes is robust. For similar reasons, they have refrained from interpreting the trends in the tropics.

Should we believe in the NDVI trend? There are no 'ground-truth' measurements of photosynthesis at northern high latitudes over the same period, and so the accuracy of the trend cannot be established unambiguously. Nonetheless, the consistency between the two independently processed NDVI datasets inspires some confidence. For corroboration, the authors present Keeling and colleagues' finding⁴ of an increasing trend in the amplitude of the atmospheric CO₂ annual cycle at Point Barrow, Alaska.

Photosynthesis removes CO₂ from the atmosphere. Carbon is returned to the atmosphere through respiration of microbes decomposing dead organic matter. The different timing in the photosynthesis and respiration gives rise to an annu-

al cycle of CO₂ in the atmosphere. Over the tropical rainforests, where inhalation and exhalation of CO₂ are large but nearly cancel over a day to a month, there is little seasonal variation in atmospheric CO₂. The largest amplitude of the CO₂ annual cycle is found at high latitudes in the Northern Hemisphere, where the growing season is short and photosynthesis and respiration are asynchronous. The positive trend in CO₂ amplitude is evidence of a consequence of increased biospheric activity, but not direct evidence of the increased activity itself. The trend in photosynthesis presented by Myneni *et al.* is thus only part of the story of the trend in CO₂ amplitude. Increased temperatures are likely to have stimulated both photosynthesis and respiration, and/or changed the phasing between the two.

What does the NDVI trend mean for the contemporary carbon budget? Again, whether the carbon inventory in the biosphere has increased or decreased over the past 15 years depends on the competition between photosynthesis and respiration. The latitudinal gradients of CO₂ and its isotopes point to a repository of anthropogenic CO₂ in the terrestrial biosphere at northern middle to high latitudes⁵⁻⁸. These inferences, on top of the NDVI and CO₂ amplitude trends, suggest that photosynthesis has won over respiration at northern high latitudes.

It will be a challenge for ecologists to explain how photosynthesis could have increased by some 10% from 1981 to 1991. Over the same period, the increase in CO₂ levels is only 4%, from 340 p.p.m.v. to 355 p.p.m.v. (parts per million by volume), and could not have enhanced photosynthesis at the NDVI rate. Temperature increases may have stimulated photosynthesis directly, or accelerated snowmelt and mobilized nutrients previously frozen in soils. The magnitudes of these effects remain to be investigated.

With the revelation, from ice-core data, of the roller-coaster fluctuations of climate, one cannot help but wonder how the biosphere has fared in the ride. Fossil pollen records show recurring rearrangements of the seating in the past. Myneni and colleagues' study provides evidence that the biosphere, at least at northern high latitudes, has been an increasingly restless passenger in recent years. Other studies⁹ suggest that the biosphere, even without rearrangement, may not be a passive passenger but may feed back on the climate change.

In 1998, NASA is scheduled to launch AM-1, the first platform of the Earth Observing System (EOS). Instruments on board AM-1 promise to be calibrated, and simultaneous observations of aerosols and other atmospheric constituents will allow for a careful removal of atmospheric

effects. What new insights into our backyards will be revealed by the forthcoming satellite observations? □

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Population biology

Hosts and parasitoids in space

H. C. J. Godfray and M. P. Hassell

Parasitoids are insects whose larvae develop on or in the body of other insects, eventually killing them (Fig. 1). Their population dynamics have been studied intensively, both because of their economic importance in controlling pests, but also because they provide a relatively simple system for investigating dynamic processes common to all consumer–resource interactions.

In recent years, theorists have been exploring how an explicit consideration of space affects the dynamic interactions between hosts and parasitoids, a move that reflects an increasing realization of the importance of spatial processes in all branches of population dynamics. But although the mathematical models reveal a fascinating menagerie of dynamic patterns, experimental studies have lagged behind, chiefly because of the great logistical problems involved. However, a series of studies, including that of Roland and Taylor on page 710 of this issue¹, have demonstrated the rewards of taking a spatial approach.

Roland and Taylor studied a moth called

Malacosoma disstria, the forest tent caterpillar, which feeds in aspen woodland in central Canada. The moth's caterpillars live gregariously in silken webs, and can become common enough to defoliate large areas of forest. Although the population dynamics are not wholly understood, it is thought that outbreaks are suppressed by parasitoids of the moth, particularly certain flies of the families Tachinidae and Sarcophagidae.

Before human intervention, aspen forests occurred over wide areas, but today they have been fragmented into stands of varying size. Roland and Taylor asked how fragmentation and host density influence the pattern of attack on *M. disstria* by the different species of parasitoid. They sampled populations of the moth and parasitoid at 127 sites within a 25×25 km grid, and repeated the exercise at 109 sites within a smaller 800×800 m grid. Employing statistical modelling techniques, they then sought to explain observed rates of parasitism by each of four species of parasitoid using two classes of explanatory variable:



Figure 1 Parasitoid attack — here a brood of the braconid wasp *Cotesia glomeratus* emerges from the caterpillar of a small white butterfly, *Pieris rapae*, before undergoing pupation.

first, the density of the host in the current and previous generations; second, a series of measures of forest fragmentation consisting of estimates of forest cover over successively largely circular areas centred at the sampling site (the estimates being obtained from satellite photographs involving GIS (Geographical Information System) technology).

Parasitism by the three largest parasitoids showed significant positive dependence on host density while the smallest species showed negative density dependence. The extent of forest fragmentation influenced parasitism by all flies. But the spatial scale at which parasitism and fragmentation were most highly correlated differed between species, with larger species being influenced by fragmentation measured over the greatest area. Moreover, whereas the three largest parasitoids showed reduced rates of attack in fragmented forests, parasitism by the smallest species was increased in small woods.

This study is significant for two reasons. First, it illustrates spatial patterns of positive and negative density dependence on a scale never previously studied. There is some controversy over exactly how density dependence in parasitoid attack influences host dynamics, and data such as these are invaluable in guiding new theory. Second, it shows how habitat change can alter host-parasitoid population dynamics. In a fragmented forest, the larger species that are probably most important in regulating the host perform poorly, something that might explain the longer outbreaks of *M. disstria* observed in such woodland. Further studies will need to try to disentangle the joint effects of the four parasitoids on host dynamics (here treated independently), as well as developing techniques for dealing with possible statistical non-independence of samples collected from a spatial grid.

In a highly fragmented landscape, individual populations may be destined to become extinct in a relatively short period of time. However, the ensemble of populations may persist if they are loosely coupled by migration and if colonization is able to balance extinction. Such a population structure is known as a classical, or 'blink-light', metapopulation.

One of the best examples of a classical metapopulation is provided by the work of Hanski and colleagues on *Melitaea cinxia*, the Glanville fritillary butterfly, in the Åland archipelago in Finland. The butterfly exists in small scattered populations in areas where its food plant grows, with each population having a relatively high probability of extinction. Previous studies had shown that extinction rates were correlated with population size, but Lei and Hanski² have demonstrated that parasitoid attack also influences extinction.

Melitaea cinxia is attacked by a parasitoid wasp, *Cotesia melitaeaeum*, which in this region appears to be specific to this host. Host populations of a particular size are more liable to become extinct if they are attacked by parasitoids. Cases of parasitoid, but not host, extinction, and parasitoid colonization of host patches have also been documented. The picture is complicated by the presence of other parasitoids in the system, including a further primary parasitoid that does best in patches where *C. melitaeaeum* is absent, and a hyperparasitoid with a very wide host range which shows a strong density-dependent response to *C. melitaeaeum* cocoons.

It is interesting that the father of parasitoid population biology, A. J. Nicholson³, anticipated host-parasitoid metapopulations 50 years ago. The Nicholson-Bailey equations, the ur-model of parasitoid population dynamics, predict unstable local population dynamics and Nicholson suggested that persistence may occur through a dynamic process of colonization and extinction exactly as in a modern metapopulation.

A more curious example of spatial processes is provided by the work of Harrison and colleagues on the moth *Orygia vetusta*, which forms persistent outbreaks on certain patches of its host plant (*Lupinus*) along the coastal strip of north California. Why do outbreaks persist in patches of contiguous bushes for many years without spreading? Experiments have excluded differences in host-plant quality, leading to the suggestion that parasitoids dispersing from the outbreak area might cause a halo of intense parasitism in the surrounding bushes that prevents the spread of an outbreak.

Earlier this year, Brodmann, Wilcox and Harrison⁴ published the results of experiments showing that parasitism is highest in the areas surrounding the outbreak, as the parasitoid hypothesis predicts. But why should parasitoids disperse away from outbreaks and so presumably lower their reproductive fitness, and why do parasitoids not reduce population densities within the outbreak? Interference between searching parasitoids is a possible solution. Like the other two studies discussed here^{1,2}, this work shows the importance of thinking spatially — but it raises as many questions as it answers. □

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Daedalus

Radical nutrition

Waste disposal is an intractable problem. Solid organic wastes give the worst trouble: paper, plastics, food residues and so on. Ideally, we should burn them to generate power. But garbage burns poorly, giving an acrid smoke. Liquid-phase combustion would be better, but needs daunting temperatures and pressures, or expensive reagents such as excited silver ions. Yet nature has two separate processes for destroying organic waste; and both use the same simple, room-temperature reagent.

Organic matter in the atmosphere is degraded by hydroxyl radicals. Generated by the action of ultraviolet light on ozone, they oxidize or decompose almost every organic molecule. And in our own bodies, the immune response attacks foreign invaders with this same deadly weapon. So Daedalus wants to recruit hydroxyl to the cause of waste disposal. The radical is perhaps most easily made by irradiating water with gamma rays, which suggests a new use for radioactive waste. But it may be wiser to make it by irradiating wet ozone or hydrogen peroxide solution with UV light.

Daedalus's pilot process illuminates an aqueous slurry of organic waste with intense UV light, and pumps in ozone or hydrogen peroxide. The waste is forcefully degraded and broken down by irresistible radical chemistry. But even the strongest UV cannot produce enough hydroxyl to oxidize tons of waste completely to carbon dioxide and water. Fortunately, it doesn't have to. Daedalus's process needs merely to degrade and oxidize the molecules of the waste sufficiently to render them soluble in water. A little oxygen forced into every molecule, breaking up long chains and terminating them with hydroxyl or carboxyl groups, should suffice.

The resulting clear soup will contain some volatiles — simple aldehydes, ketones, and the like — which could be distilled off for use as solvents. The residual solution will contain the involatiles: long-chain fatty acids, polyfunctional alcohols, saccharides, and so on. The mixture, says Daedalus, should be quite nutritive.

Even DREADCO's ever-optimistic Marketing Department has small hope of selling partly oxidized rubbish as a human food product. Fortunately, the practice of feeding rubbish to animals is already well established. So Daedalus will incorporate his oxidized rubbish into pet foods, cattle feed, and so on. Hydroxyl radicals are utterly deadly to viruses and bacteria, so these products will be quite sterile. There is no danger of another BSE crisis.

David Jones

Edward M. Purcell (1912–97)

Pioneer in nuclear magnetic resonance and in radioastronomy

Edward Mills Purcell died on 7 March 1997 at his home in Cambridge, Massachusetts, from respiratory failure. The last time he participated in a scientific meeting was in December 1995, when the Golden Jubilee of the demonstration of nuclear magnetic resonance (NMR) in condensed matter was celebrated at Harvard University. Fifty years earlier, Purcell, H. C. Torrey and R. V. Pound had observed the magnetic resonance absorption of hydrogen nuclei in paraffin (caused by a transition from one spin state of the nucleus to another), for which Purcell shared the 1952 Nobel prize for physics with Felix Bloch. The latter had led a group at Stanford University, which independently and almost simultaneously detected nuclear induction in water.

Purcell and Bloch first met, and first discussed NMR, at the April 1946 meeting of the American Physical Society in Cambridge, Massachusetts. They realized that they were studying the same physical phenomenon, albeit with somewhat different methods. While there was a spirit of healthy competition, their cordial relations are apparent from the telegram that Purcell received hours after the announcement of the Nobel prize, "I think it is swell for Ed Purcell to share the shock with Felix Bloch".

A year earlier, Purcell and Harold I. Ewan had discovered that the microwave emission from atomic hydrogen in our Galaxy corresponds to the atom's hyperfine transition at 1,420 MHz (also a consequence of nuclear spin). This experiment was carried out on a shoestring budget. A small, horn antenna pointed out of a window on the top floor of the Lyman Physics Laboratory at Harvard University, and a microwave receiver at a wavelength of 21 cm measured the variation in the effective radiation temperature of the Milky Way as it rotated overhead past the aperture of the fixed horn. In the last year of his life, Purcell confided that he considered this and later contributions to radioastronomy at least as significant as his NMR work.

Edward Purcell was born on 30 August 1912, in Taylorville, Illinois. His father worked for a local telephone company, and as a high school student Ed tinkered

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with discarded telephone equipment and perused issues of the Bell Systems Technical Journal. He enrolled as an electrical engineering student at Purdue University, where he assisted a faculty member in a project on electron diffraction, and so acquired a taste for physics. He spent a year as an exchange student in Karlsruhe, Germany, before going to Harvard for a PhD working on a spherical electrostatic mass spectrometer, under the guidance of K. T. Bainbridge.

In the fall of 1940, the MIT radiation laboratory was established to further the development of radar technology. I. I. Rabi, the associate director, soon invited Purcell to join, and he became the head of the scientific development group in 1942. He was involved with the successes of X-band radar at a wavelength of 3 cm, and also learned that K-band radar, at 1.25 cm, had not been a military success because of strong absorption by water molecules in the atmosphere.

In the summer of 1945, the scientists of the radiation laboratory could all turn their attention to the question of what physics to do in peacetime. The problem of nuclear magnetic resonant absorption presented itself. Transitions between nuclear spin levels in a magnetic field had been studied in molecular beams, but hadn't been seen in solids. Purcell, Torrey and Pound were still engaged full time at the radiation laboratory, but during evenings and weekends they assembled equipment around a large electromagnet at Harvard, which had been used for cosmic-ray research in pre-war days. They borrowed a radio-frequency generator and receiver, and built a resonant cavity filled with paraffin. They observed proton magnetic resonance on 15 December 1945. Purcell described in his Nobel lecture how he had gained a new insight into the

natural world: passing heaps of snow on his way home after the discovery, he wondered about all the protons precessing quietly in the Earth's magnetic field.

In February 1946 Purcell accepted me as a laboratory assistant. He, Pound and Torrey were still employed full time writing volumes of the MIT radiation laboratory series. Having spent the war in the Netherlands, occupied by Hitler's forces, I now had the good fortune to find myself in the right place at the right time. Purcell was most helpful in getting me started — he knew what it was like to be a foreign exchange student from his days in Germany. Purcell, Pound and I concentrated on problems of nuclear magnetic relaxation. We introduced the concept of motional averaging of local magnetic fields, which results in extremely sharp resonances in fluids. We did not foresee the widespread applications that were to follow, including the use of high-resolution NMR spectroscopy in chemical analysis and the development of magnetic resonance imaging in medical diagnostics.

In the 1950s and early 1960s, Purcell served on a number of high-level government committees. As a member of the Presidential Scientific Advisory Committee he twice made a presentation to President Eisenhower on the issues of space technology and space exploration. He was happier, however, when he could again devote all his professional time to teaching and research. He wrote a well-known textbook on electricity and magnetism for a Berkeley physics series. He was artistic, and carefully prepared his own drawings and figures. He did theoretical and experimental work on the search for a magnetic monopole, analysed the motion of interstellar dust particles and studied the locomotion of bacteria in fluids — which resulted in the popular lecture and publication *Life at High Reynolds Number*.

From 1960 on, Purcell no longer wanted to be responsible for a laboratory or graduate students. He preferred to discuss broad scientific issues with colleagues, and work out the details on his own. He was a deep thinker and valued his private life. He was modest and never actively sought recognition, although he enjoyed receiving the honours that were deservedly bestowed on him. It is a privilege to have known him as a person and as a scientist.

Nicolaas Bloembergen

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A prokaryotic phytochrome

Phytochrome photoreceptors are almost certainly ubiquitous in green plants, regulating numerous aspects of development throughout their life cycle. Phytochromes were thought to exist only in plants, but the recently described sequence of the chromosome from the cyanobacterium *Synechocystis* revealed a gene that seemed to encode a phytochrome-like protein¹. By expressing this gene in *Escherichia coli* and feeding appropriate chromophores we show that it encodes a phytochrome, which may offer an excellent starting material for crystallization and X-ray diffraction analysis.

The carboxy-terminal amphiphilic structure of phytochrome resembles that of bacterial sensory histidine-kinases, a group of enzymes used by prokaryotes to monitor and react to various aspects of their environment²⁻⁴. Conceptual translation of the *Synechocystis* sp. PCC 6803 open reading frame slr0473 (the putative *Synechocystis* phy gene) yields a product that shows similarity to plant phytochromes throughout its length and to bacterial sensory kinases towards the C terminus^{1,5}. In particular, the chromophore-binding domain, highly conserved in all phytochromes, is clearly represented in the product (residues Val 246–Asp 280). This, however, does not prove that the gene product is a genuine phytochrome. Phycocyanin levels prevent spectral photoreversibility measurements^{6,7} of phytochrome in cyanobacteria, so we investigated the puta-

tive phy gene product by expression-cloning in *E. coli* using the vector pQE12.

Expression of the *Synechocystis* PHY apoprotein was very efficient. Products of relative molecular mass 85,000 accumulated to about 50% total soluble protein (Fig. 1a, lane 1). This contrasts with results from similar approaches with plant phytochrome genes in *E. coli* which are usually very weakly expressed or give rise to largely insoluble products⁸⁻¹⁰. The clone was engineered to express a C-terminal polyhistidine tag for nickel-affinity purification. The product bound quantitatively to Ni-NTA (lane 2) and was eluted as a homogeneous apoprotein (lane 3), which could be concentrated to a 5–10 mg ml⁻¹ solution.

Plant phytochrome apoproteins autocatalytically attach linear tetrapyrrole chromophores such as phycocyanobilin (PCB)¹¹, abundant in the cytoplasm of cyanobacteria. Indeed, the *Synechocystis* PHY apoprotein attached purified PCB, producing visibly photochromic holoprotein (phy*, Fig. 1b). In contrast, plant phytochromes expressed in *E. coli* show poor autoassembly, folding incorrectly^{8,9,12}. *Synechocystis* phy* was analysed spectrophotometrically after exposure to saturating monochromatic 657 nm (red) and 731 nm (far-red) irradiation (Fig. 1c). The spectra are reminiscent of plant phytochrome-PCB adducts¹¹ with absorbance maxima at 658 and 702 nm after red and far-red irradiation, respec-

tively, and an isosbestic point at 677 nm.

This is the first report of a spectrally functional prokaryotic phytochrome. In most lower organisms light is detected by retinal or coumaric acid-based photoreceptors. However, a *Fremyella* gene, *rcaE*, involved in chromatic adaptation and encoding a putative histidine-kinase sensor protein was recently described¹³. Although the conceptual gene product shows local similarities to PHYE in *Arabidopsis*, no significant homology to the phytochrome chromophore-binding domain is apparent¹³ and photoreceptor activity has yet to be demonstrated. The *Synechocystis* phy gene product is the most phytochrome-like of the *Synechocystis* genome and among all known prokaryotic sequences.

A simple prokaryote has advantages for basic studies of phytochrome biology. For example, co-expression in the heterologous *E. coli* host might be useful in studying subsequent components of the signal transduction pathway. Furthermore, milligram amounts of homogeneous, spectrally active *Synechocystis* phytochrome holoprotein can be produced easily in our system. As concentrations of 10 mg ml⁻¹ can be achieved readily — at least ten times higher than in other phytochrome overexpression systems known to us — there remains no barrier in principle to obtaining crystals for X-ray diffraction analysis of phytochrome molecular structure.

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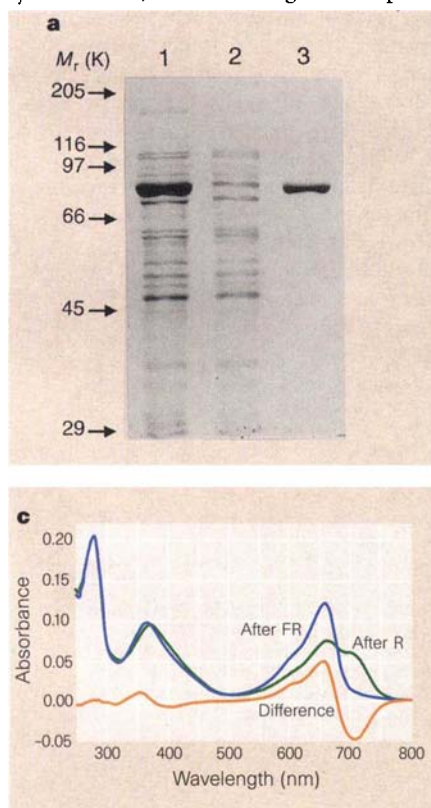


Figure 1 a, Expression and affinity purification of recombinant PHY apoprotein in *E. coli*. SDS-PAGE 10% gel stained with Coomassie. Lane 1, total soluble protein in lysate; 2, after adsorption to Ni-NTA matrix; 3, 250 mM imidazole eluate. b, Photochromicity of phy* holoprotein. Stoichiometric amounts of PCB were added to PHY (3 mg ml⁻¹). After autoassembly (20 min in darkness) the sample was divided and each portion irradiated with 731 nm (far-red, left) or 657 nm (red, right) light. Note the blue or green transition associated with phytochrome photoconversion. c, Absorbance characteristics of phy* after irradiation with saturating red (R) or far-red (FR) light, and the calculated difference spectrum.

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Circadian clock in Malpighian tubules

Circadian clocks impose daily rhythms on bodily functions. They have been identified within the central nervous systems in a wide range of animals, from invertebrates to humans, suggesting that circadian time-keeping in animals is achieved by the central nervous system. We have now found that *Drosophila* Malpighian tubules contain a circadian clock that is autonomous from the brain. Temporal integration in *Drosophila* may be regulated by a collection of independent clocks rather than one central pacemaker.

Studies of *Drosophila* have led to the identification of two clock genes, *per* (period) and *tim* (timeless), which produce circadian fluctuations of their messenger RNAs and their encoded proteins (Per and Tim)¹⁻⁴. This time-keeping mechanism involves a Per-Tim heterodimer, which mediates rhythmic transcription of both genes through a negative feedback loop⁵. The clock has been mapped to the brain, because rhythmic and nuclear expression of Per in specific brain regions is essential for the imposition of daily rhythms on behaviour⁶. However, *per* mRNA and protein also occur rhythmically in the abdomen^{3,7}.

We have investigated the expression of Per and Tim in *Drosophila* Malpighian tubules — non-innervated epithelial tubes comprising large secretory cells involved in urine excretion. By staining the secretory cells of wild-type adults with antibodies to either Per or Tim, we have revealed daily cycles in the expression of clock proteins (the staining was absent in the tubules of null mutants, *per*⁰ and *tim*⁰; data not shown).

To test the role of the brain in the rhythmic expression of clock proteins in the Malpighian tubules, we removed the heads from adult flies and compared the patterns of expression of the proteins Per and Tim in intact and decapitated flies. In the 12-hour light–12-hour dark cycle (LD 12:12) there was a clear rhythm in the level of clock proteins in both intact and decapitated flies (Fig. 1, upper panel). Per and Tim were abundantly present in the nuclei of all secretory cells towards the end of the dark period, but we did not detect them in the tubules towards the end of the light period. This rhythm closely resembles the rhythm reported for the brain, but the two are independent, because the rhythm in the tubules persisted in headless flies.

To test whether the cycling of clock

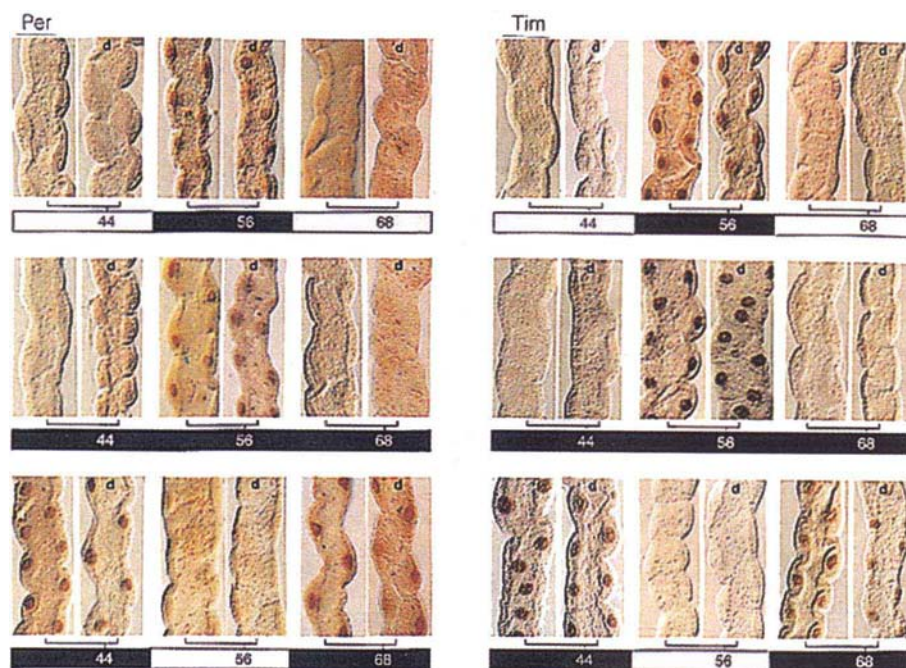


Figure 1 Immunodetection of Per and Tim proteins in Malpighian tubules of intact or decapitated (d) flies. Flies kept in LD 12:12 were decapitated before the lights were turned off and placed in the following regime: normal LD 12:12 photoperiod (top), constant darkness (middle) and reversed light–dark (bottom). The number of hours in each regime before dissection is indicated on the photoperiod bar. Per and Tim cycling continued in constant darkness and was phase-shifted in reverse light–dark in both control and decapitated flies. Antibodies against Per provided by J. C. Hall and R. Stanewsky, and against Tim by A. Sehgal and M. Hunter-Ensor. Antisera were visualized with the vectastain ABC kit.

proteins in the tubules represented a true circadian clock, we subjected intact and decapitated flies to either constant darkness or light–dark phase shifts. The cycling of Per and Tim persisted in complete darkness in both intact and decapitated flies (Fig. 1, middle panels). Furthermore, when decapitated flies were exposed to a 12-h shift in their photoperiod, the Per and Tim rhythms in their tubules were also reset by 12 hours, just as in the intact flies (Fig. 1, lower panels). Thus, tubules exhibit free-running cycling of clock proteins, the rhythm of which can be reset by light, in the absence of both brain and eyes.

We have previously identified a peripheral clock in moth testes⁸. Our results from *Drosophila* show that the peripheral clock uses the same molecules and mechanisms as the brain-located clock. It is not surprising that tissues simpler than the central nervous system have pacemaking ability, as bacteria and simple eukaryotes exhibit circadian rhythms. As complex animals evolved from simple organisms, simple tissues could have retained their rhythm-generating capacity rather than surrender to the central brain clock.

The question remains as to what the function of the Malpighian tubule clock might be. Adult flies have behavioural rhythms that may require periods of higher excretory activity (associated with

increased metabolic rates). Fluid excretion in fly tubules is driven by transmembrane ion movements. Ion pumps, such as vacuolar ATPase, operate in this tissue⁹, so a circadian clock in the tubules may affect the rates of ion movement across the cell membranes — as in other circadian systems¹⁰. This clock, which occurs in fly Malpighian tubules and can be manipulated experimentally, may thus provide a model to explore the links between circadian mechanisms and membrane physiology.

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Carbon isotope evidence for early life

The Letter by Mojzsis *et al.*¹ on evidence for life on Earth before 3,800 million years ago was accompanied by a discussion of the effects of prograde thermal metamorphism on carbon isotope ratios. This discussion included an analysis of the effect a Rayleigh distillation process might have in reducing the ¹³C/¹²C ratio of organic carbon that is residual to oxidation during diagenesis and metamorphism. The calculations presented in that discussion (including Fig. 3, its caption and text on page 58 of ref. 1) contained errors that contributed to the conclusion that it would have been physically impossible for such a process to produce the observed $\delta^{13}\text{C}$ values of approximately -35‰ (with respect to the PDB standard) from initial abiotic values of -10‰ . Here we correct those errors and discuss the significance of the correct calculation.

Two related errors are present in the Rayleigh distillation calculation as published in ref. 1. First, the Rayleigh equation in the caption of Fig. 3 uses $\delta^{13}\text{C}$ values where ¹³C/¹²C ratios should be used. Second, the direction of the equilibrium isotopic fractionation between CO₂ and graphite is opposite to that necessary for the use of the Rayleigh equation as it is commonly derived for stable isotopes, and its absolute magnitude differs substantially from that estimated by experimental studies. The correct form of the Rayleigh equation for the distillation of CO₂ from residual graphite is:

$$R_f = R_i \times F^{(\alpha-1)} \quad (1)$$

where R_i is the isotope ratio ¹³C/¹²C in the graphite before oxidation and distillation of CO₂, R_f is the same ratio in the residual graphite after this reaction, F is the mole fraction of the residual phase (graphite) remaining after this reaction and α is the equilibrium isotope fractionation factor between the evolved CO₂ and residual graphite at any given step in the Rayleigh distillation process, defined as $\alpha = R_{\text{CO}_2}/R_{\text{graphite}}$ (ref. 2). Translation of equation (1) into standard δ notation yields:

$$\delta_f = (1000 \times (F^{(\alpha-1)} - 1)) + \delta_i F^{(\alpha-1)} \quad (2)$$

where δ values are defined by the equation:

$$\delta = (R/R_{\text{std}} - 1) \times 1000 \quad (3)$$

with R_{std} being the ¹³C/¹²C ratio of a reference standard (for example PDB).

The correct value for α (CO₂–graphite) at 400 °C, the temperature appropriate for modelling prograde

metamorphism of the samples in question, is 1.0111 (ref. 3), corresponding to an 11‰ difference in $\delta^{13}\text{C}$ between CO₂ and graphite. Using this value for α , an initial $\delta^{13}\text{C}$ value of -10‰_{PDB} (the lower limit of 'abiotic' carbon from ref. 1), and equation (2), a $\delta^{13}\text{C}$ value of -35‰ in residual graphite are obtained when $F=0.1$ (10% of the original carbon remains in the rock as graphite), substantially different from the value of $F=2.5 \times 10^{-11}$ estimated by Mojzsis *et al.*¹

It was argued in ref. 1 that the extreme value of F that was calculated disproved the possibility that low $\delta^{13}\text{C}$ was the result of oxidation during metamorphism because virtually no carbon would remain in the rock. The correct value for $F=0.1$ (90% reaction) is not sufficiently extreme to rule out the possibility, and instead the calculation permits that, under the assumed conditions, the oxidation of carbonaceous matter during metamorphism could produce residual graphite with $\delta^{13}\text{C}$ values in the range that is often regarded as diagnostic of biogenic carbon (less than -20‰).

The debate over the carbon-isotope shifts that are expected to accompany diagenesis and metamorphism of carbonaceous matter is old (for example, refs 4, 5), and controversy as to the origin and initial isotopic composition of carbon in Archean rocks continues⁶. Resolving this issue for a given sample requires that the action of oxidation reactions that proceed by Rayleigh distillation during hydrocarbon maturation and metamorphism is proved or disproved. The requirements for such a process to lead to low $\delta^{13}\text{C}$ residual carbon are that fluids in equilibrium with residual hydrocarbons and/or graphite must have a high ratio of CO₂/CH₄, and that isotopic exchange between residual hydrocarbons and/or graphite and fluids must be rapid. Under common oxygen fugacity (f_{O_2}) conditions and mid-crustal pressures, and at temperatures less than 500 °C, C–O–H fluids are generally CH₄ dominated⁷. Thus, these requirements are not expected to be met unless metamorphic conditions are unusually oxidizing or if temperatures are higher⁷.

A number of previous studies have demonstrated that metamorphism of carbonaceous matter in sedimentary rocks commonly leads to increases, rather than decreases, in $\delta^{13}\text{C}$ owing to the distillation of CH₄ and exchange with high $\delta^{13}\text{C}$ carbonate minerals^{8,9}. Armouring of carbonaceous matter by crystals of a C-poor phase, such as is the case for the graphite analysed by Mojzsis *et al.*¹, would tend further to reduce the opportunities for isotope fractionation during metamorphism¹⁰. For these reasons the authors of the original paper¹ support the initial interpretation

that measured values of $\delta^{13}\text{C}$ in graphite define maximum limits on the initial $\delta^{13}\text{C}$ values of precursor carbonaceous matter.

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Clouds, precipitation and temperature range

The daily range of surface air temperature has decreased worldwide since the 1950s, with an increase in night-time minimum temperature (T_{min}) exceeding the increase in daytime maximum temperature (T_{max})^{1–3}. Coincident increases in total cloud cover found in many locations¹ have been cited as a plausible cause^{1,4,5}. Clouds have a damping effect on diurnal temperature range (DTR), aiding cooling of the Earth's surface by reflecting sunlight and heating the surface by increasing downward longwave radiation, as confirmed by model studies^{6,7}. Increasing aerosol concentrations have been suggested as a possible cause of the observed increase in cloudiness⁴, but we suggest that greenhouse gas-induced increases in thick precipitating clouds and precipitation are better candidates to explain the decrease in DTR.

DTR has been negatively correlated with both total cloud cover and precipitation throughout this century, over all regions for which data are available (Fig. 1). In particular, the correlation is remarkable for the long-term trends, especially over mid-latitude Canada and Australia. Available surface and ship observations⁸ indicate that, during the 1952–81 period, cumulonimbus, nimbostratus and cirriform clouds increased substantially over Australia, Europe and the United States, but there was little change in low-altitude cumulus and stratus clouds over northern mid-latitude oceans. Consistent

with the cumulonimbus and nimbostratus increase, clouds with low bases showed the largest increase from 1948–87 over the United States⁹. However, available data are insufficient to determine whether thin cumulus and stratus clouds have also increased over the United States.

Satellite observations (Fig. 2), which are good for studying clouds with high tops, show that monthly anomalies of only optically thick and high-topped clouds are strongly correlated with precipitation anomalies over a 66-month period of coincident observations. As the correlation between cloud cover and precipitation is independent of the timescale of variation¹⁰, the correlated secular trends of precipitation and cloud cover shown in Fig. 1 indicate that it was the thick precipitating clouds that increased during the past 4 or 5 decades.

Thick precipitating clouds such as cumulonimbus and nimbostratus have low cloud bases, which radiate back to Earth at temperatures close to ground temperature, and high cloud tops with large column-integrated condensate which reflect back most of the sunlight. These two properties make these clouds very effective in damping DTR, consistent with a general circulation model (GCM) study⁷. Over the contiguous United States and Europe, the correlation between DTR and precipitation is substantially higher than that between DTR and total cloud cover (Fig. 1), suggesting that DTR decreases are more likely to be related

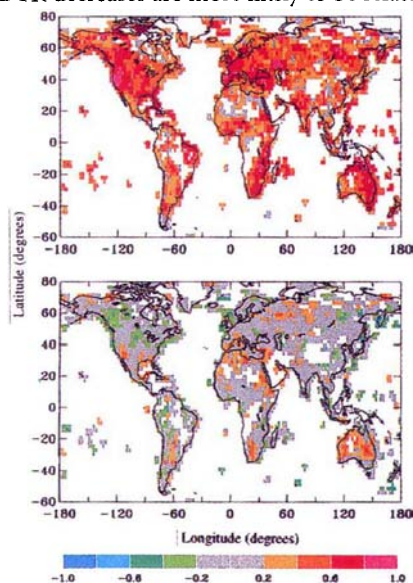


Figure 2 Maps of correlation coefficients (-1.0 to $+1.0$) between surface observed monthly precipitation anomalies and the ISCCP¹⁵ cloud cover anomalies of nimbostratus, cirrocumulus/cirrostratus and deep convective clouds (upper panel) and other types of clouds (lower panel). Seasonal variations were removed by subtracting out the monthly means. The systematic error in ISCCP clouds due to changes of satellites was corrected. The data are for July 1983 to December 1988.

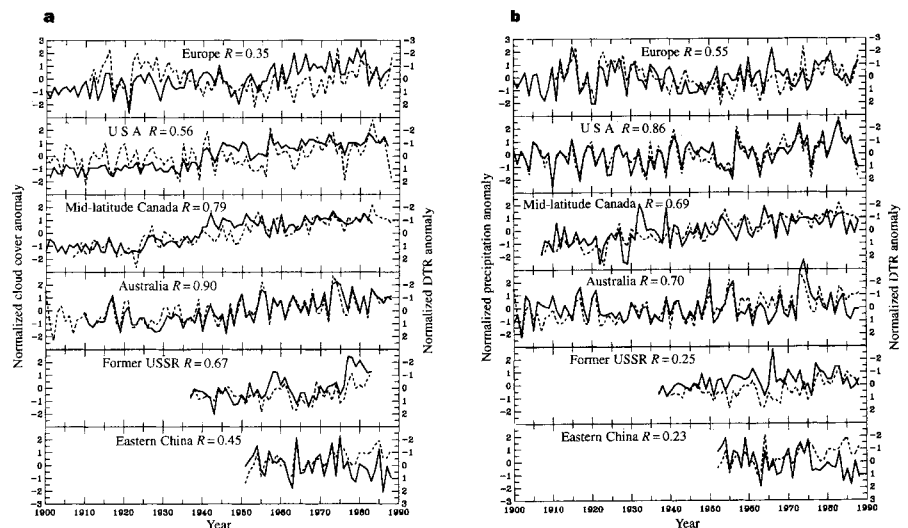


Figure 1 Variations of annual anomalies of diurnal temperature range (dashed line) with a, total cloud cover^{11–14}, and b, precipitation (solid lines), over various regions during this century. Note that the scale for DTR anomalies on the right side decreases upwards. The DTR data are derived from the NOAA National Climatic Data Center GHCN v2 data set. Correlation coefficient R between the curves is given.

to increases in precipitating clouds.

Given the high correlation between DTR and precipitation (Fig. 1), the increased precipitation itself may have contributed to the DTR decrease through surface evaporative cooling (especially over dry areas). $T_{\max}$ is more sensitive to water availability than $T_{\min}$ owing to the existence of an unstable surface layer (thus higher turbulent mixing) and larger potential evapotranspiration during the day⁹. This mechanism is likely to be most effective from late summer to autumn when soil moisture levels are low in northern mid-latitudes, consistent with the seasonal signal of DTR changes, which show largest decreases in the northern autumn^{1,3}. The mechanism should also work under clear-sky conditions, which is in agreement with the finding that the DTR of clear skies has decreased in the United States⁵, although increased greenhouse gas concentrations (including water vapour)² may also contribute to the DTR decrease. (GCM results⁶ suggest that the direct effect of industrial aerosols on DTR is small compared with the concurrent CO_2 forcing.)

Because anthropogenic aerosols may increase the concentration of cloud condensation nuclei and reduce mean cloud-droplet sizes, thus inhibiting precipitation, the increases in precipitation and precipitating clouds are likely to be related to other forcings such as the enhanced greenhouse effect². The increases in precipitation and precipitating clouds are consistent with GCM experiments with increasing CO_2 , which generally produce increased precipitation (mostly in winter) and increased convective cloud amounts over northern mid-latitude land areas². Simulated changes in mid-latitude nimbostratus, a dominant precipitating cloud there, are more model-dependent by comparison and therefore not conclusive.

Consistent with our argument, model simulations⁶ with increasing CO_2 concentrations reproduce DTR changes comparable to the observations through mechanisms similar to those mentioned above. However, more detailed analyses of changes of cloud types are needed before a definite conclusion can be reached.

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Shrinking minds and swollen heads

The Encyclopedia of Psychiatry, Psychology, and Psychoanalysis

edited by Benjamin B. Wolman

Holt: 1996. Pp. 649. \$135

The Blackwell Dictionary of Neuropsychology

edited by J. Graham Beaumont, Pamela

M. Kenealy and Marcus J. C. Rogers

Blackwell: 1996. Pp. 788. £80

V. S. Ramachandran and J. J. Smythies

At a time when behavioural science, psychology and neuroscience are becoming increasingly fragmented into narrow specialisms, there is a real need for single-volume compilations that provide brief, informative summaries for the nonspecialist. These two volumes were presumably produced with this objective in mind. Unfortunately, the quality of the entries varies considerably, with some so banal as to take one's breath away.

Consider these penetrating insights from the *Encyclopedia*. Love: "It became apparent that increases in liking do not always lead to love, especially romantic love." Laughter: "The research of developmental psychologists has contributed most to our understanding of laughter, although by and large their conclusions are not unequivocal. It is clear that laughter matu-

rationally precedes humor appreciation."

Pair-bonding: "When limerence (infatuation) is at its peak, the limerent person undergoes subjective and somatic changes similar to those that signify a state of expectancy or anticipation. The cycle of sleeping and waking is altered, as is food intake, thermoregulation, and kinesis. At the approach or thought of the beloved, there are changes in pulse rate, blushing, breathing, swallowing, perspiration and vocal fluency. Communication with the beloved becomes a prime occupation. Dreams may involve images of being together romantically, erotically, genitally. They may culminate in orgasm with the partner represented in absentia."

Acculturation: "The assimilation of a newcomer into a foreign culture is highly dependent on the development of understanding between the recent arrival and his or her hosts. Crucial to the development of such understanding is effective interchange of information between the new arrival and his hosts, that is, intercultural communication."

There is very little in these descriptions that one's grandmother would not have known. They are a sad reminder that the behavioural sciences today are hardly more advanced than physics was in the seven-

teenth century, or biology was in the nineteenth. So the fault, perhaps, is not so much with the *Encyclopedia*, but with the field as a whole. Perhaps we should not expect too much from a field that is still in its infancy.

Anyone interested in the history of ideas would be puzzled by the following striking differences between advances in biology and advances in psychology. The progress of biology has been characterized by landmark discoveries, each of which resulted in a breakthrough in understanding — the discoveries of cells, Mendel's laws of heredity, chromosomes, mutations, DNA and the genetic code. Psychology, on the other hand, has been characterized by an embarrassingly long sequence of 'theories', each really nothing more than a passing fad that rarely outlived the person who proposed it.

Why the difference? There are at least four possible reasons. First, it may be that the profession attracts especially dull people — this seems possible, but unlikely. Second, it may be that human behaviour is inherently more complex, capricious and difficult to fathom than biology — there is obviously some truth to this view. Third, as pointed out by Peter Medawar, the difference may reflect the fact that psychologists suffer from "physics envy" — they assume tacitly that the only sure way to progress is to imitate the mannerisms, fads and fashions of the physical sciences, including the belief that a detailed quantitative study of any phenomenon is intrinsically meritorious.

A good example of this fallacy is the 100-year history of IQ research — the attempt to provide a single-number evaluation of 'intelligence', an entity (or multiple entities really) about which little is known. Among the many acrimonious battles fought in psychology, none is more absurdly comical than the so-called 'nature-nurture' debate about intellectual ability. It is significant, and a sign of the difference in maturity of the two fields, that no such 'debate' exists in biology. Everyone recognizes the complementary roles of genes and the environment, but no conferences are held to decide 'which is more important'. Most biologists recognize that intelligence is not one trait but probably several dozen abilities influenced by many different genes each affecting more than one character. And before we can even begin to assess the relative roles of nature and nurture, we need to understand what these traits are, and what the underlying mechanisms might be.

Sure enough, IQ tests can be used as a rough and ready rule of thumb for estimating 'general intelligence' when time is short (as when recruiting for the Navy, for instance), just as pulse, blood pressure and



Chilling tales of winters past

Fairs were held on the frozen Thames in London in past centuries. This picture shows the river at Tower Bridge on 11 February 1895 when the ice was not firm enough for a fair but navigation had almost ceased. From

Frosts, Freezes and Fairs: Chronicles of the Frozen Thames and Harsh Winters in Britain since 1000 AD by Ian Currie (Frosted Earth, £8.95). The book contains many early photographs and engravings.

temperature could be regarded as providing a reasonable estimate of a patient's general health. Yet the key difference is that medicine has emerged from the dark ages and progressed rapidly to the point where we no longer use only pulse and temperature, but have more than a dozen tests for liver function alone. Psychology, on the other hand, is still stuck with IQ.

Another problem with the 'inheritance' of intelligence — also not addressed by these books — is that any mental 'trait' is the result of extraordinarily complex interactions between multiple genes and multiple environmental variables. So it may be no more meaningful to ask 'which is more important, nature or nurture?' than to ask 'which contributes more to the wetness of water, the hydrogen molecules or the oxygen molecules?'

More specifically, the extent to which even a single gene influences intelligence is itself a function of the particular numerical value chosen for a given environmental variable. The fact that a significant part of the variance in IQ does seem to depend on genes (as measured by twin studies, for example) may have more to say about the monotony of American homes and elementary schools than about the relative roles of nature and nurture. The critical environmental variables simply may not have been identified. We mention the IQ debate only because we found no critical evaluation of any of these issues in either volume.

The fourth reason for psychology's singular lack of progress stems, we believe, from an excessive reliance on metaphorical explanations as substitutes for a genuine understanding of underlying neural mechanisms. Dreams, for example, are said to arise from a need "to fulfil unconscious wishes". Laughter? Why, to "discharge psychic energy", of course. Why do anything at all? To reduce "cognitive dissonance". (Medawar calls explanations of this kind "analgesics" because "they dull the ache of incomprehension without removing the cause".) Nor are the black-box diagrams of cognitive psychologists any better, because they usually do not have the foggiest notion of what each box is actually doing. Metaphors are often a good place to start, but, sooner or later, one would like an explanation that does more than explain just the phenomenon in question.

One needs to look for answers in the structure and function of the nervous system. As an analogy, consider the history of research on heredity and genetics, where complex macroscopic phenomena (why are offspring a curious blend of their parents?; why don't goats give birth to pigs?; what are genes?) eluded our understanding until the discovery of DNA. Ironically, it was the unravelling of the double-helical structure of this molecule that completely 'explained'

heredity and genetics. It was as though the structural logic of DNA dictates the functional logic of heredity.

It is unlikely that some such general unifying principle exists in psychology or neuroscience, but nothing prevents us from looking for structural explanations of at least certain aspects of our minds. In this regard, the *Dictionary* is a much more useful compendium; it contains several superb essays, such as the ones on 'blind sight' attention, autism, amnesia, hemiplegia, blood flow studies, agnosia, hippocampus and face recognition, to mention just a few.

The *Encyclopedia*, on the other hand, devotes only 16 out of 606 entries to the neurobiological aspects of psychology and psychiatry, of which five are devoted to antidepressant drugs and three to a strange account of the biochemistry of learning and memory that is based mainly on Hyden's long-extinct RNA theory. And, except for the one good entry on "Psychoanalysis and the brain" (in which D. O. Hebb and neural networks get their only mention), there is no discussion of the neuroscientific basis of the major psychoses, the neuroses, neural computation, information processing, artificial intelligence, attention, motivation, mood, and almost every aspect of what is now by far the most interesting, influential and important current work on psychology. Yet the book finds space to expound the views of several obscure nineteenth-century psychologists and early psychoanalysts.

The book is, in fact, an encyclopaedia of psychoanalysis and some aspects of (mainly social) psychology. Any future printing should be retitled to reflect this emphasis, and even then the book is unlikely to have much impact outside a 20-mile radius of Manhattan. □

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The cultures of genetics

La Souris, la Mouche et l'Homme

by François Jacob

Editions Odile Jacob: 1997. Pp. 237. FF120

Benno Müller-Hill

Genetics is a young science that is growing up quickly. It now moves so fast that the public are really scared: what are they to make of genetic engineering, or cloning? In this climate of fear, more than ever before, books are needed to explain to the public what genetics is all about. One may naively think that any bright professor of genetics could do that, but history has shown it is not that easy. The experts are usually rather narrow mind-

ed; they understand their own small corner of genetics but are unable to paint the broader picture the public need to see.

If ever a book was written that achieved these aims, this is it. The author, François Jacob, is a member of the French Academy. He won the Nobel Prize for Physiology or Medicine in 1965 with Jacques Monod and André Lwoff for work on the operon theory of gene control. He is one of the few scientists who write comprehensibly and eloquently. In the past 27 years he has published three books, all of which are good, but this seems to be his best. It is written in French, but deserves to be translated into many languages.

It begins with a chapter on prediction, which is the essence of all scientific theory. Jacob tells us, and I will not here divulge the erotic details, how the greatest Greek soothsayer, Tiresias, learned to predict. There is even a brief description of Dolly, the famous cloned sheep, which Jacob must have inserted the day before the book went into print!

In the next two chapters, *Drosophila* and the mouse are presented as objects of genetic research. Jacob tells the full story from Thomas Morgan to Christine Nüsslein-Volhard and Eric Wieschaus. Embedded in this are reminiscences of the centennial of Gregor Mendel in Brno in 1965 and a brief description of Stalin's attempt to destroy genetics through the charlatan Trofim Lysenko. Jacob then discusses the difficult shift from *Escherichia coli* to mouse at the Institut Pasteur. We learn the dictum of Charles DeGaulle: "There are three things in France which one should not touch: The Collège de France, the Institut Pasteur and the Tour Eiffel". Jacob thus comments on Georges Pompidou who tried to stop Monod becoming the director of the Institut Pasteur: "Pompidou, like all presidents of the Republic, had the memory of an elephant and the thirst for revenge of a rhinoceros". The mouse chapter has just one small omission, which could easily be put right in a new edition: there is no mention of either general or conditional knockout of mouse genes, which gave us deeper insight into how they work.

The next two chapters, on the modular structure of proteins and chromosomal DNA, show how pieces of old solutions are used again and again in evolution. In the last two chapters, on "The Good and the Bad" and "The Beautiful and the True", Jacob takes the position that science cannot and should not decide what is good or bad. These values have to come from elsewhere. Jacob presents the criminal horror produced by German human geneticists under Hitler. With regard to science he concludes: "One has to tell the truth and only the truth", and "We are condemned to never-ending research".

What makes this book so readable? It is not just that the science is described so clearly. It is the mix of scientific fact and history

with Greek mythology, European literature and art, with metaphors the general reader will understand. For example, Jacob points out that the term 'biology' was invented in almost the same year that Goethe's *Werther*, the first novel culminating in suicide, was published. It is just wonderful to read about genetics and to be reminded of details from the classics one has almost forgotten. If there were more books like this, genetics might not be under such an attack as it is now. It would be part of European culture. □

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Digging up the past

Eyewitness to Discovery

edited by Brian M. Fagan
Oxford University Press: 1997. Pp. 493.
£19.99, \$39.95

The Oxford Companion to Archaeology

edited by Brian M. Fagan
Oxford University Press: 1997. Pp. 844. £35, \$55

Warwick Bray

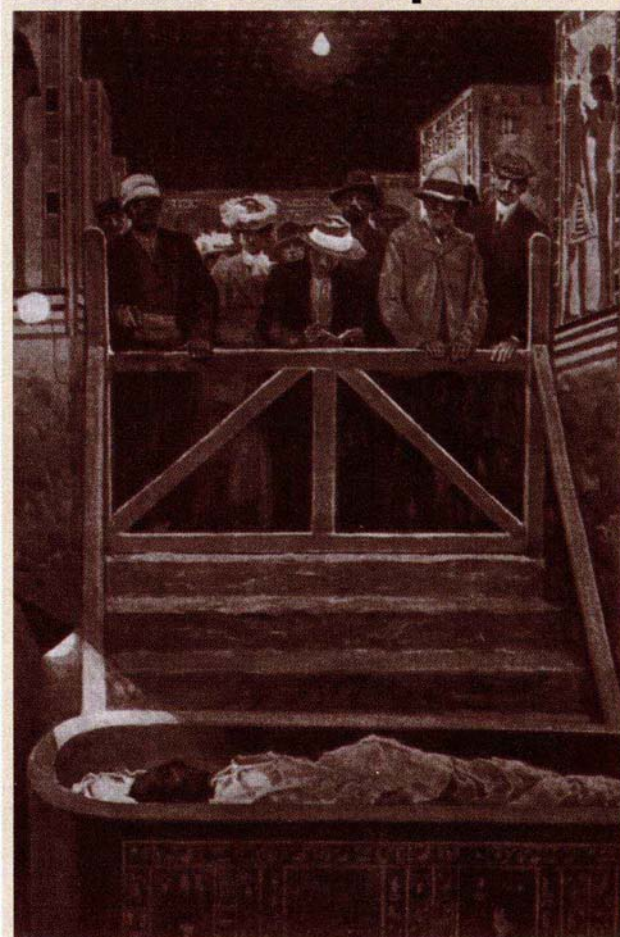
Brian Fagan is one of the most successful popularizers of archaeology, and has a reputation for being both interesting and accurate. He is also, as these two books show, an enterprising editor.

Eyewitness to Discovery gathers together 55 first-person accounts of archaeological discoveries, each prefaced by a short biography of the writer and an indication of why the finds are significant. There are the usual all-time favourites — Ur of the Chaldees, Macchu Picchu, Otzi the Ice Man, Great Zimbabwe, Tutankhamun's tomb, Heinrich Schliemann's disingenuous account of his work at Troy. And there are several important but little-known excavations — a Pleistocene cave in Tasmania, a sixteenth-century death by ice collapse in Alaska, a seventeenth-century Dutch outpost in the South African Cape. All the authors can write, some of them supremely well, and their stories are still fresh and lively today.

My favourite is Raymond Dart's account of the first *Australopithecus* skull, the Taung baby, which arrived at his house just as he was dressing for a wedding. Any *Nature* reader who has had to weigh domestic harmony against the urge to finish an experiment will know exactly how Dart felt as he stood there, collar in one hand and the brain of a completely new hominid in the other.

But there is more than mere entertainment here, and Fagan sets out to educate as well as to amuse. The accounts by Boucher de Perthes and Arthur Evans of finding Palaeolithic hand-axes in the Somme grav-

An audience with a pharaoh



Tourists at the turn of the century viewing the mummy of Amenophis II in an exhibit arranged by the archaeologist Howard Carter. Egypt's Valley of the Kings is of course still visited by millions of tourists, who now have a new guidebook available to them. *The Complete Valley of the Kings: Tombs and Treasures of Egypt's Greatest Pharaohs* by Nicholas Reeves and Richard H. Wilkinson (Thames and Hudson, £19.95, \$29.95) surveys the art, archaeology and history of the place. It includes an account of the recent discovery of the burial chapels of Ramesses the Great's many sons. There are more than 500 illustrations.

els make the point that archaeology in Europe has its roots in the stratigraphic geology of the nineteenth century. In America, by contrast, the closest links are with anthropology. From the twentieth century, the account of the Koster site in Illinois shows the archaeologists counting pollen grains, phytoliths and carbonized seeds, epitomizing the modern era of scientific excavation and emphasizing that today's archaeologists dig for information, not for treasure.

The most recent extract in the book dates from the 1990s, when the discovery of an eighteenth-century Black cemetery in downtown Manhattan sparked a conflict involving developers, city officials, contract archaeologists and the Black community. Archaeology is no longer just about 'digging things up'.

The *Oxford Companion* is a weightier tome altogether, with more than 800 pages, 700 signed articles and 372 authors (the address list is a reference tool in itself). The scope is global, but with the occasional touch of marketing chauvinism: the British Isles get a general essay, France and Germany do not. All the expected categories are covered: sites, artefacts (though not many), famous archaeologists, cultures and regions, techniques of investigation,

raw materials, technology, and the theoretical concepts that underpin the discipline.

The match between authors and topics is impressive. Fagan has somehow persuaded top archaeologists to write about what they know best. Sites are described by their excavators, scientific techniques by their inventors or by leading practitioners, and so on. Many of the essays are provided with short bibliographies.

Individual entries are long enough to say something worthwhile, and the ones I can check are up to date and accurate. Inevitably, there are gaps. To find out about the history of glassworking, I followed the index to an excellent survey of Roman glass, but there the trail petered out. Nor are the key words of essay titles always predictable. "Hieroglyphs" are followed by "High altitude occupations in the American West" and by the equally surprising "High technology" before the sequence returns to orthodoxy with "Hillforts" and "History of archaeology". Even using the index it is not always easy to find one's way around, but the rewards of browsing are all the greater for being unexpected.

The *Oxford Companion* is aimed at students and serious professionals as well as the general public, and has strong entries dealing with ideas, political issues and

intellectual principles, besides basic facts. There is discussion of all the major 'isms' of archaeology — structuralism, processualism, post-processualism, neo-Marxism, critical theory, systems theory, cognitive archaeology, cultural ecology, and so on. These accounts prove that, contrary to much popular mythology, the underlying assumptions are usually quite simple and can be expressed in

straightforward English. The volume has no party line, and authors are allowed to speak their minds.

The entry on "Ethics in archaeology" asserts, correctly, that "archaeology is a political endeavour as well as a science", and then explains why. Elsewhere we are told that the future of feminist archaeology "remains ambiguous and full of tensions between different theoretical orientations",

that collecting antiquities encourages looting and that the art market has much to answer for, and that popular fiction portrays archaeologists as "shallow, secretive, hysterical and, worst of all, subject to self-interest". Now where could that idea have come from? □

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In retrospect chosen by Keith Devlin

Syntactic Structures

by Noam Chomsky
(1957)

The publication of Noam Chomsky's *Syntactic Structures* marked a watershed in the study of human language. It was *Syntactic Structures* (SS) that turned linguistics from a branch of anthropology into a mathematical science.

At a mere 114 pages, it is perhaps more accurately described as a pamphlet than a book. Published in 1957 in the Netherlands by the little known publishing house Mouton, partly at Chomsky's expense, it was number four in a series called "Janua Linguarum". It has not been out of print since.

Although the techniques developed in SS have long been abandoned by linguists — including Chomsky himself — it is fair to say that all present-day linguistics has its origins in SS, even parts of linguistics that began with attempts to refute Chomsky's theories.

The first clearly recognizable attempt to develop linguistics as a science of language was made by the Swiss scholar Mongin-Ferdinand de Saussure in the early years of the twentieth century. The book that bears his name, *A Course on General Linguistics*, published after his death in 1916, is generally regarded as the first textbook in modern linguistics.

In many respects, US linguistics began with the work of Leonard Bloomfield a few years later. Unlike de Saussure, Bloomfield was convinced that linguistics should become a mathematical science, a position for which he actively campaigned. Among those who were influenced by Bloomfield's argument was the linguist Zellig Harris at the University of Pennsylvania. It was to Harris that the young Avram Noam Chomsky (born in 1928 in Philadelphia) turned for advice as a graduate student at the university in the 1940s.

Leaving Pennsylvania university in 1951, Chomsky spent a period as a junior fellow in philosophy at Harvard, where in 1955 he completed his doctoral dissertation on "Transformational Analysis". The same year, he obtained a position at Massachusetts Institute of Technology, where he has remained ever since.

Syntactic Structures is a portion of Chomsky's doctoral dissertation. As you might infer from the delay in publication of SS, coupled with the obscure choice of publisher,

the linguistics community was initially somewhat dismissive of his work — it did not seem to fit in with what anyone else was doing. Indeed, it certainly did not fit in. It was the start of something very new and very different.

Often in science, the key to a major breakthrough is to recognize what is the right question to ask, given the scientific tools available at the time. The fundamental question Chomsky asked in SS was: how can we describe the abstract structure of a grammatical sentence? More precisely, what are the relationships that must hold between the words that make up a grammatical sentence, but which do not hold for an ungrammatical sequence of words?

Since native speakers of a language do seem to agree, to a remarkable degree, about what constitutes a grammatical sentence, the question undoubtedly has scientific merit. But how could one go about trying to find an answer? From a theoretical point of view, the number of grammatical sentences is infinite — you can keep on creating longer and longer sentences by joining any two sentences by the word 'and'. So the question cannot be answered by listing all the grammatical sequences of words.

The question is also, at first sight, a rather odd one, in that it has nothing to do with whether the sequence of words makes sense. You can sequence words that have that sense but are not grammatical sentences — like this sequence. On the other hand, Chomsky's own famous example, "Colorless green ideas sleep furiously", shows that you can have a completely nonsensical sequence of words that everyone agrees is a grammatical sentence. What Chomsky realized was that it was possible to separate off sentence structure — that is, syntax — from both meaning (semantics) and use (pragmatics), and still have a phenomenon that could be meaningfully studied. Indeed, it was precisely by isolating syntax that Chomsky was able to bring the exact methods of mathematics to bear on syntactic structure.

Specifically, it was Chomsky's familiarity with the techniques of twentieth-century mathematical logicians such as Alan Turing and Kurt Gödel that provided the key to his new science of syntax. Logicians had formulated rules that describe how propositions may be combined to form a valid proof. Chomsky set out to formulate rules that describe how words

may be put together to form a grammatical sentence. It was, in other words, an 'axiomatic' approach to language. He called his rules (or 'axioms') for syntax a 'grammar'.

The introduction to SS begins: "Syntax is the study of the principles and processes by which sentences are constructed in particular languages. Syntactic investigation of a given language has as its goal the construction of a grammar that can be viewed as a device of some sort for producing the sentences of the language under analysis. More generally, linguists must be concerned with the problem of determining the fundamental underlying properties of successful grammars. The ultimate outcome of these investigations should be a theory of linguistic structure in which the descriptive devices utilized in particular grammars are presented and studied abstractly, with no specific reference to particular languages."

Just as the mathematicians of the late nineteenth century regarded the formulation of an appropriate set of axioms as the pinnacle of understanding, so too Chomsky put great emphasis on the formulation of a grammar. This is made clear by the following crucial sentence from chapter 6 of SS: "A grammar of the language L is essentially a theory of L."

Despite his initial success, in subsequent years Chomsky's work came in for a great deal of criticism. But, criticism aside, what Chomsky did was to put on the table a collection of precisely defined, testable hypotheses about syntax. Before Chomsky, linguistics had been entirely a soft science, concentrating largely on the description of various linguistic phenomena. Chomsky's theory of syntactic structure was very definitely hard science in the sense of Karl Popper. Linguistics has not been the same since.

Today, with a rapidly growing interest in the science of mind, linguistics is increasingly finding itself playing an important role, by providing a scientifically precise tool for observing the mind. Whether for or against Chomsky's theories and proposals, no linguist could deny that this new-found application of the science of language is yet another part of the legacy of *Syntactic Structures*.

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Mechanisms of angiogenesis

Werner Risau

After the developing embryo has formed a primary vascular plexus by a process termed vasculogenesis, further blood vessels are generated by both sprouting and non-sprouting angiogenesis, which are progressively pruned and remodelled into a functional adult circulatory system. Recent results, particularly from the study of mice lacking some of the signalling systems involved, have greatly improved our understanding of the molecular basis underlying these events, and may suggest new approaches for treating conditions such as cancer that depend on angiogenesis.

When Leonardo da Vinci first speculated about the heart and circulatory system (see *Nature* **380**, 9; 1996), he could only analyse the mechanisms of organ formation by analogy, so he suggested that the vasculature developed like a tree from a seed (the heart) by sprouting roots (the liver capillary meshwork) and a trunk with major branches (the aorta and arteries) (Fig. 1). Although sprouting of blood vessels is indeed a principal mechanism of blood vessel formation, termed angiogenesis, other mechanisms are now known. Rather than sprouting from the heart, the vascular system is laid down before the heart starts beating. Conversely, adult blood vessels generally form from pre-existing vessels in direct response to tissue demands.

Here I summarize our present understanding of blood vessel formation and raise some outstanding questions. Figure 2 shows the sequence of events discussed; more detailed reviews of vasculogenesis and angiogenesis can be found elsewhere¹⁻³.

Vasculogenesis

The early vascular plexus forms from mesoderm by differentiation of angioblasts (vascular endothelial cells that have not yet formed a lumen), which subsequently generate primitive blood vessels. The molecular mechanisms responsible for this process, termed vasculogenesis⁴, are emerging (Fig. 2). Mesoderm-inducing factors of the fibroblast growth factor family are crucial in inducing paraxial and lateral plate mesoderm to form angioblasts and haematopoietic cells. The existence of a bipotential precursor for these cell types (the so-called haemangioblast) is suggested by defects in both the haematopoietic and angioblastic lineages of embryos lacking vascular endothelial growth factor receptor-2 (VEGF-R2, also called Flk-1 and KDR in mice and humans,

respectively; Fig. 2)⁴. But only haematopoietic stem cells are affected^{5,6} in embryos lacking the transcription factor *Scl/tal-1*. After differentiation, VEGF-R2 is downregulated in haematopoietic but not in endothelial cells.

The other receptor for VEGF, VEGF-R1 (Flt-1), plays a role later, as mice lacking VEGF-R1 produce angioblasts, but their assembly into functional blood vessels is impaired⁷. VEGF itself acts in a paracrine manner as it is produced by the endoderm, whereas its receptors are expressed by mesoderm-derived angioblasts. Threshold VEGF levels seem to be needed to maintain angioblast differentiation, as mice lacking one copy of the VEGF gene die *in utero*, with aberrant blood vessel formation in the yolk sac and embryo^{8,9}. The different phenotypes of mice lacking VEGF and VEGF-R2 suggest that either maternal VEGF or another VEGF-R2 ligand (possibly VEGF-C) is active during early mesoderm induction¹⁰, but both the VEGF receptors and sufficient levels of their ligands are necessary for vasculogenesis. Induction of VEGF-R2 may thus initiate angioblast differentiation, whereas the quantity and activity of VEGF ligands determines angioblast survival. Intraembryonic angioblasts derived from the splanchnopleuric mesoderm can produce haematopoietic cells, unlike those derived from the somatopleuric mesoderm¹¹, indicating that subtypes of angioblasts may exist. Rather than forming blood vessels where they originate, some angioblasts migrate over long distances¹², forming a vascular plexus at a distant site (such as the perineural vascular plexus). Unanswered questions in vasculogenesis include the nature of the signals (other than mesoderm-inducing signals) that induce expression of the VEGF receptors and their ligands; and whether a bipotential haemangioblast exists, and, if so, what determines its commitment to the angioblastic or haematopoietic lineage?

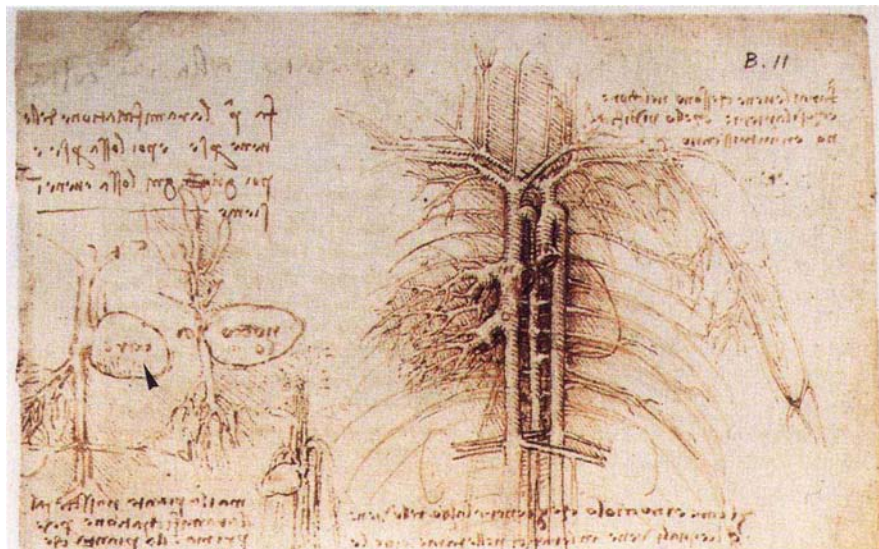


Figure 1 Analogy between the botanic and the vascular tree as drawn by Leonardo da Vinci (taken from 'the anatomy of man: the cardiovascular system' (ca. 1508)). The drawing is annotated by notes in his trademark cryptic mirror-writing, for example the big seed of the left plant labelled 'core' (heart) is indicated by the arrowhead.

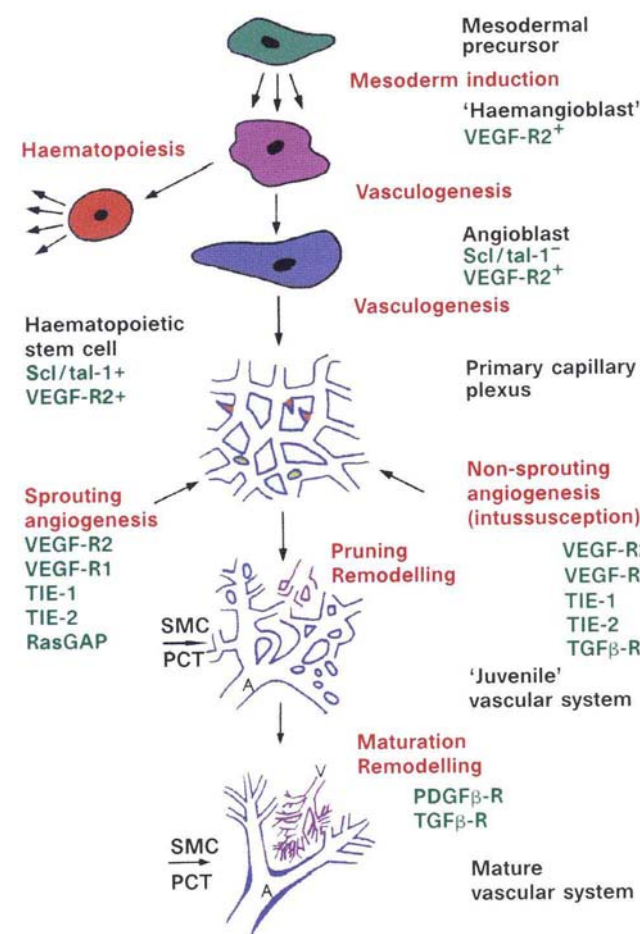


Figure 2 The processes (red labels), molecules (green labels) and appearances (black labels) involved in vascular development. Red tips in the primary capillary plexus represent sprouts, yellow circles represent splitting pillars (Fig. 3). The haemangioblast as a bipotential precursor is still hypothetical and intermediate steps between some processes have been omitted. Remodelling and maturation is dependent on the tissue and organ context. It is schematized here from observations in the avian yolk-sac vascular system. A, arteriole; V, venule; SMC, smooth muscle cells; PCT, pericytes. None of the figures is drawn to scale.

Sprouting and non-sprouting angiogenesis

After the primary vascular plexus is formed, more endothelial cells are generated, which can form new capillaries by sprouting or by splitting from their vessel of origin in a process termed angiogenesis. There are at least two different types: true sprouting of capillaries from pre-existing vessels, and non-sprouting angiogenesis or intussusception (Figs 2 and 3).

Sprouting angiogenesis occurs both in the yolk sac (Fig. 3) and in the embryo (most frequently during later organogenesis, particularly in the brain). Proteolytic degradation of the extracellular matrix is followed by chemotactic migration and proliferation of endothelial cells, formation of a lumen and functional maturation of the endothelium. Almost all known angiogenesis-activating factors induce one or more of these activities in endothelial cells *in vitro*, but it is unclear which factors act *in vivo*¹³. One may be VEGF, as it is an endothelial-specific growth and chemotactic factor. In the embryonic brain, secreted isoforms of VEGF are produced in the periventricular layer of the neural tube, which may direct the sprouting of neural capillaries¹⁰. These sprouts also exhibit long extensions of the endothelial cell at the sprout tip directed towards the VEGF-producing cells of the periventricular layer, which are hallmarks of sprouting angiogenesis.

Non-sprouting angiogenesis is a process of splitting pre-existing

vessels by transcapillary pillars or posts of extracellular matrix¹⁴, first described in the embryonic lung¹⁵ (Figs 2 and 3). Concurrent sprouting and non-sprouting angiogenesis are involved in the vascularization of organs or tissues such as the lung, heart and yolk sac during development (Fig. 3). *In vivo*, non-sprouting angiogenesis can occur by proliferation of endothelial cells inside a vessel, producing a wide lumen that can be split by transcapillary pillars, or fusion and splitting of capillaries. The type of angiogenesis in a given organ or tissue may depend on the number of vessels already present when the organ starts to grow rapidly. Non-sprouting angiogenesis predominates in the lung, for example, which contains intrinsic endothelial precursors and is initially vascularized by vasculogenesis¹⁶, whereas sprouting angiogenesis is used in the brain anlage, which does not contain angioblasts.

Targeted mutations in mice indicate some of the mechanisms responsible for both processes. Early embryonic vessels (the vascular plexus formed by vasculogenesis) typically contain plump endothelial cells forming wide lumina without a correspondingly thick vascular wall, which may reflect VEGF's ability to induce formation of larger vessels by enhancing fusion of capillaries. (Indeed, VEGF-deficient embryos lack even the dorsal aorta^{8,17}.) The endothelial receptor tyrosine kinase TIE-2 or TEK is also necessary for sprouting. In TIE-2 knockout mice, capillaries do not sprout into the brain, leading to death around embryonic day 10, and blood vessels in areas such as the perineural plexus, where the lumina are normally small, exhibit a persistently wide lumen¹⁸. TIE-2 thus seems to modulate VEGF activity, inducing endothelial cells to narrow their lumina for sprouting or splitting¹⁸, and the pattern of signalling by the VEGF and TIE receptors may direct both sprouting and non-sprouting angiogenesis^{7,18,19}. The identification of TIE ligands, called angiopoietins, will improve our understanding of their role^{20,21}. However²², different molecular mechanisms must be involved in vasculogenesis and angiogenesis, as TIE receptors are required only for the latter.

Pruning and remodelling

The emerging vascular plexus is rapidly remodelled to resemble a mature system with larger and smaller vessels (Fig. 2). The process responsible is termed 'pruning' as the resulting pattern resembles a tree²³, but the molecular mechanisms remain obscure.

Although excess endothelial cells generated by vasculogenesis before circulation begins are lost, there is no evidence that the process involves apoptosis. This may reflect either the methodology used to detect apoptotic cells or their rapid disappearance²⁴. In any

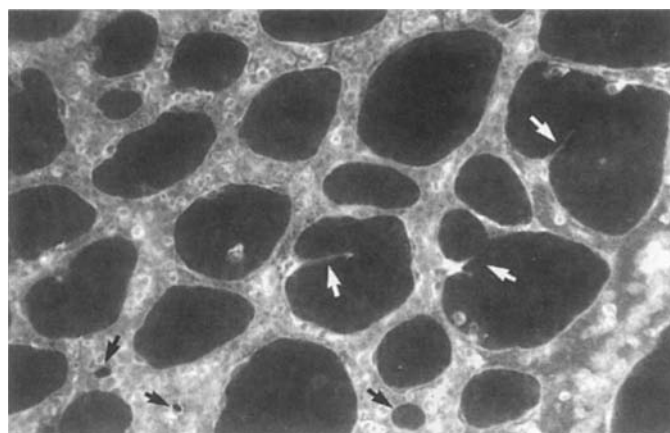


Figure 3 Sprouting and non-sprouting angiogenesis in the 3-day-old quail yolk sac. Both processes occur simultaneously (black arrows point to the intussusceptive pillars and white arrows indicate the long tips of sprouts in immunofluorescence using QH1 monoclonal antibodies).

case, the lethal effect of VEGF heterozygosity points to a dose-dependent effect of VEGF on endothelial cell survival. VEGF is a survival factor for the endothelial cells in the embryonic retina in which pruning was first described: hyperoxia inhibits VEGF expression, leading to regression and death of retinal vessels, which can be rescued by intravitreal injection of the factor²⁵. It is unclear, however, whether hyperoxia induces blood vessel regression under physiological conditions.

'Pruned' endothelial cells may also reassemble into other vessels or dedifferentiate. Angioblasts are migratory, and endothelial cells destined to become dorsal aorta endothelium can participate in brain angiogenesis when transplanted into the brain¹². Endothelial cells have only been shown to de-differentiate during development of the heart valves, in which endocardial cells migrate into the cardiac jelly²⁶, and it is unclear if this process occurs elsewhere. These results suggest that although endothelial cells are plastic during embryonic and early postnatal development and can participate in sprouting and non-sprouting angiogenesis as well as vasculogenesis, they may also die when deprived of survival factors.

Endothelial cell fate is thus determined by the combined effects of positive and negative signals simultaneously transduced by numerous receptors. Endothelial cells in the perineural plexus of a ten-day-old mouse embryo, for example, express high levels of VEGF-R1, VEGF-R2, TIE-1 and TIE-2, while their ligands are expressed in surrounding tissues. The mere presence of ligands and receptors does not prove that the receptors are activated, however, and the temporal and spatial activation of the different receptors will be of interest. How does the endothelial cell interpret these signals so as to remodel the perineural plexus and invade the neuroectoderm while maintaining contact with neighbouring endothelial cells and keeping cell polarity? How is the spacing between invading sprouts determined? Most of the molecular pathways involved in this remarkable process remain unknown.

The phenotype of mice lacking the Ras GTPase-activating protein (GAP) suggests that it is a key effector for the receptors involved²⁷. Remodelling of the vascular plexus in the yolk sac is delayed, and the primary plexus is not reorganized into a normal vascular system. In the embryo proper, the dorsal aorta is thin and forms aberrant ventral branches, whereas chimaeric embryos that survive longer exhibit severe oedema and a grossly abnormal vasculature.

Maturation and remodelling

Both intra- and extraluminal factors are involved in the further maturation of blood vessels (Fig. 2), which mature or regress along with the tissue or organ they supply. In developing vascular beds, the direction of flow can change, with arterioles becoming venules and vice versa. Non-perfused blood vessels regress. Similarly, in the corneal model of tumour angiogenesis, a bunch of sprouts initially invades the avascular cornea, but only one artery and one vein persist once they have reached the implanted tumour. Better perfusion of a tissue may lead to hyperoxygenation and thus to vessel regression, a mechanism that may also render periarterial but not perivenous spaces avascular. Although initially independent of the circulation, the vascular system is later shaped by forces generated by the circulation. Shear stress, for example, strongly affects endothelial cells, inducing modification of cell-cell as well as cell-extracellular-matrix junctions and upregulating growth factors such as platelet-derived growth factor (PDGF)-BB (refs 28 and 29). Simultaneously, the vascular wall matures: the basal lamina is modified, pericytes and smooth muscle cells differentiate and elastogenesis begins in elastic arteries.

At some time between postnatal life and adulthood, survival of endothelial cells in most tissues (except, for example, fenestrated endothelium³⁰) becomes independent of VEGF. Early studies indicated that proper maturation required interactions of endothelial cells with themselves, extracellular matrix or other cells (such as smooth muscle cells or pericytes). Recent studies of mutant mice

show that adhesion molecules such as fibronectin and the α_5 and α_v integrin receptors play a crucial role in vasculogenesis and angiogenesis (reviewed in ref. 31; R. Hynes, personal communication), suggesting that they are required for endothelial maturation and differentiation. Similarly, transforming growth factor (TGF)- β is very important for proper vascular development in both the embryo³² and the adult, as inactivation of the TGF- β signalling system leads to telangiectasia, a vascular malformation reminiscent of defects in vascular remodelling and maturation³³. Another molecule with an early essential role in vascular development is tissue factor, a procoagulant receptor on embryonic endothelium somehow involved in recruiting perivascular cells³⁴, contact between which triggers expression of TGF- β , inhibiting endothelial cell proliferation *in vitro*³⁵. Later in embryonic development, PDGF seems to play a similar role. PDGF- β is upregulated by shear stress in endothelial cells, and activates the PDGF- β receptor expressed on perivascular mesenchymal cells, pericytes and smooth muscle cells. Mice lacking either molecule die *in utero* from defective vascular maturation, apparently caused by the lack of cells attracted and attached to endothelial cells (C. Betsholtz, personal communication). Finally, angiogenic inhibitors may be differentially or combinatorially involved in vascular maturation. Many involve proteolysis³⁶ (for example of plasminogen to angiostatin³⁷), and may affect embryonic vascular development.

Remodelling thus appears to be initiated by circulating factors and other signals such as shear stress and hyperoxia that are transduced by endothelial cells. Increased shear stress, for example, increases PDGF secretion from endothelial cells, attracting neighbouring perivascular cells expressing the PDGF receptor and leading to their attachment to endothelium. This in turn activates TGF- β , which may induce alterations in the extracellular matrix that stabilize the phenotype of endothelial cells, as well as inhibiting their proliferation (and possibly rendering them independent of VEGF). The precise mechanisms leading from the angioblast to the quiescent, organ-specific and established endothelium of the adult remain to be identified.

Regulation of angiogenesis

Physiology, pathology and *Drosophila* all provide insight into the control of angiogenesis. Metabolic demands are thought to regulate the vascularization of tissues and tumours. Hypoxia upregulates VEGF^{38,39}, owing both to increased transcription mediated by hypoxia-inducible factor-1 (HIF-1) and an increase in VEGF mRNA stability dependent on the 3' region of the mRNA⁴⁰. The molecular machinery initiating angiogenesis may thus be driven by deprivation of oxygen and nutrients such as glucose, which regulate gene expression in an overlapping pattern⁴¹. In haemangioblastomas, this pathway appears to have gone awry. Loss of the von Hippel-Lindau tumour-suppressor gene, for example, stabilizes VEGF mRNA in the absence of hypoxia⁴²⁻⁴⁴.

The tracheal system in *Drosophila* offers a precedent for oxygen-regulated gene expression during embryonic development⁴⁵. Unlike mammals, *Drosophila* has a poorly developed vascular system, but an extensive tracheal system which undergoes branching morphogenesis comparable to the vertebrate vascular system. Like the vascular system, it is initially genetically determined by genes such as *trachealess* and *breathless*, but is later extended according to oxygen needs. *trachealess* is homologous to HIF-1 (ref. 46), suggesting that members of this family are involved in similar early events. An oxygen-responsive gene, *pruned*, is also responsible for the final branching pattern⁴⁷. Although *pruned* encodes a transcription factor related to the vertebrate serum-response factor, its vertebrate homologues do not seem to regulate vascular endothelial genes (A. Nordheim, personal communication). But vascularization of vertebrate neural tissue appears to be regulated by hypoxia-induced VEGF expression in both retinal astrocytes and the ventricular cells of the embryonic retina and forebrain^{10,48}. So whereas genetic

control of vasculogenesis is exerted by factors related to *trachealess*, metabolic control may depend on angiogenic factors reflecting tissue demands.

Adult angiogenesis

Despite their low turnover, adult endothelial cells are not post-mitotic but can form new blood vessels. The mechanisms underlying physiological angiogenesis during the ovarian cycle and pathological angiogenesis in tumours, ophthalmic and rheumatic diseases³ are not yet fully understood. In pathological conditions and in wound healing, angiogenesis is a complex process accompanied by, and possibly requiring, inflammation. Nevertheless, those factors known to be necessary for adult angiogenesis also regulate embryonic angiogenesis. Indeed, VEGF, first found to affect angiogenesis in malignant gliomas³⁹, also regulates angiogenesis in many different tumours, and inhibition of VEGF signalling inhibits both tumour angiogenesis and the growth of experimental solid tumours^{49–51}. But the mechanisms responsible for vascularization of tumours growing *in situ*, rather than as a transplant under the skin of mice, are still poorly understood. Although the sequence of events superficially resembles that in embryonic angiogenesis, the additional events in an established quiescent vessel are probably necessary, involving cell-cycle progression, remodelling of cell adhesions and junctions, induction of proteolytic activities, and neutralization of inhibitors.

Recent work has emphasized genetic switches regulating angiogenesis⁵², but the detailed genetic changes and their effects on angiogenesis must still be identified. Oncogenic, tumour-suppressive or mutant forms of mitogenic signalling pathways and cell-cycle control switches probably regulate genes involved in angiogenesis³, but whether they act directly or by way of the changing micro-environment in a growing tissue mass remains unclear. Malignant cells with mutations in the *p53* gene, for example, can survive hypoxic conditions that kill less malignant cells⁵³, indicating that the supply of nutrients and oxygen to a growing tumour can affect its growth, malignancy and metastatic potential. An improved understanding of normal angiogenesis may suggest new approaches to therapy. □

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Surprisingly rapid spreading of newly formed intermediate waters across the North Atlantic Ocean

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Ocean temperature, salinity and chlorofluorocarbon concentration data are used to track the recent spreading of cold intermediate-depth water masses from the Labrador Sea across the northern North Atlantic Ocean. These water masses, which are formed from surface waters by deep convection in the Labrador Sea, spread three to four times faster than previously estimated, with associated consequences for the North Atlantic thermohaline circulation.

The northern North Atlantic Ocean plays a key role in determining long-term changes in the climate system. At the northern subtropical latitudes the oceans carry approximately half of the total meridional heat flux required by the global radiation budget, and 60% of that is accomplished by the overturning cell of the North Atlantic¹. The enormous transfer of heat to the atmosphere from northward-flowing surface waters and the subsequent loss of buoyancy results in convective formation of intermediate- and deep-water masses.

In the central Labrador Sea, deep convection in late winter creates the relatively homogeneous Labrador Sea Water (LSW) with its unique characteristics of low salinity, low vertical density gradient, high oxygen content and high anthropogenic tracer concentrations such as chlorofluorocarbons (CFCs). These signals can be traced at depths between 1,000 and 2,000 m as the LSW spreads laterally into other regions. LSW follows three primary pathways away from the formation region^{2,3} (Fig. 1): northeastward into the Irminger basin, eastward near 50° N latitude with several bifurcations east of the Mid-Atlantic Ridge (MAR), and southward as part of the Deep Western Boundary Current.

The wintertime formation of LSW is not a process of constant intensity or of constant characteristics every year. Hydrographic time series of the central Labrador Sea reveal that deep convection varies on decadal timescales⁴. Two periods of intensified LSW production were identified during the past 30 years, from 1972 to 1976, and from the late 1980 (ref. 5) onwards (Fig. 2). Owing to the lack of appropriate observations, there is some ambiguity in defining the onset of the recent convection period. The first indication of its onset came from single-station CFC measurements in the south central Labrador Sea in July 1986. From 1990, LSW was found to be significantly cooler, denser and thicker in the entire central Labrador Sea than during the past 30–40 years (Fig. 2). Convective LSW formation reached maximum intensity in 1992 and 1993. The first indication of significant LSW changes outside its source region was found in 1991, in the subpolar North Atlantic, by comparison with historical data⁷, but these changes were not compared with the ongoing change in LSW formation at source. The time dependence of LSW formation is reviewed in ref. 6. Age estimates of LSW^{7–10} found in various areas of the northeastern Atlantic show that the year of the formation (the 'vintage') varies from 1972 to 1986.

Based on a new set of temperature–salinity (*T/S*) and CFC data, we report rapid changes of intermediate-depth water–mass characteristics observed between 1990 and 1996. We are able to trace the propagation of a series of new modes of LSW throughout the northern North Atlantic, and we are also able to link these modes to a series of intensifying deep wintertime convection events after 1988 in the central Labrador Sea. The appearance of these LSW vintages in other regions of the ocean is marked by substantial cooling, deepening and densification of the local LSW core. Using the *T/S* and CFC data independently, the trans-Atlantic travel time from the source region to the eastern boundary is estimated to be 4–5.5 years. This indicates a surprisingly high mean speed of about 1.5–2 cm s^{−1}. Our estimate exceeds those previously suggested^{7,8,10} by about three to four times. This exceptional cooling of intermediate waters, with the attendant consequences for the thermohaline circulation of the North Atlantic, is still in progress.

The '1988 Labrador Sea Water cascade'

Our observations, obtained as contributions to the World Ocean Circulation Experiment (WOCE), are along three hydrographic sections (Fig. 1) and span the period 1990–96. To trace the changes of LSW properties we observe hydrographic characteristics at the salinity minimum of the LSW core (Fig. 3). This straightforward water-mass-following method is insensitive to depth or density variations of the LSW vintages in a similar way to the potential vorticity minimum method².

The temporal development of LSW core temperatures reveals an annual cooling in the Irminger Sea of the order of -0.28°C in 4 years (Fig. 4a, Table 1). We call this multi-step event the '1988 LSW cascade'. Cooling in the Iceland basin of -0.19°C , and in the Rockall trough area of -0.11°C , in this period also indicates renewed LSW. Although we find a cascade-like signal in the Iceland basin, in the Rockall trough area, the signal of newly ventilated water appears only in the 1994 data, indicating a later arrival of the 1988 LSW cascade at the eastern boundary. Substantial cooling results in an increase of density (Fig. 4b) and in a deepening of the core layer, which are largest in the Irminger Sea (change in potential density anomaly $\Delta\sigma = 0.018\text{ kg m}^{-3}$ and change in depth, measured in pressure units, $\Delta p = 240\text{ dbar}$). A striking but not surprising feature (Fig. 4a) is the splitting of the LSW core layer into two different hydrographic regimes west and east of the Reykjanes ridge (at

31° W). This splitting reflects the effects of the longer pathways into the eastern basins and enhanced mixing over topography.

The characteristic property changes of the 1988 LSW cascade are cooling, densification and deepening, but only minor (or no) freshening (Table 1). These results correspond well with observations in the central Labrador Sea. From 1988 to 1994, the LSW formation was characterized by a temperature decrease of the order of -0.4°C , without significant salinity changes and accompanied by a density increase of $\sim 0.05\text{ kg m}^{-3}$ (Fig. 2). This offset is in contrast to the properties generated during the preceding formation period (1972–76), where strong cooling was accompanied by a substantial freshening of the order of -0.08 practical salinity units, p.s.u., and consequently only very small densification.

Spreading across the sub-polar gyre

In Fig. 5a we compare potential-temperature/salinity (θ/S) values for LSW (from the central Labrador Sea) with those from the Irminger Sea. The Labrador Sea observations are grouped in tight clusters representing the homogeneous (θ/S) signature of each LSW vintage, whereas the Irminger Sea values are more scattered and generally higher. This demonstrates the influence of mixing along the spreading path. However, all data are in the same density range and in both basins they show the same step-wise increase in density with time since the early 1990s. As we found no indications of deep convection in the Irminger Sea, we interpret this layer as being a direct intrusion of LSW which spreads from the source region along its isopycnals (surfaces of constant density). The alignment of core values along isopycnals suggests that the annual intrusion of LSW into the Irminger Sea originated in the Labrador Sea during the convection season in March of the same (or the previous) year. For the 1993 vintage in the Labrador Sea data we cannot show the counterpart in the Irminger Sea, because there are no observations

available. Also, owing to a lack of appropriate Labrador Sea data in 1991, it is not clear whether the 1990 or the missing 1991 vintage are linked to the 1991 Irminger Sea data. Finally, the June 1995 Irminger Sea cluster shows the same signature as observed in December 1994, indicative of a minimum travel time exceeding 3 months.

We conclude that the intrusive events observed in the Irminger Sea are a result of convection in the Labrador Sea of the same year, resulting in a maximum time lag of 6 months. (Our CFC observations—discussed below—allow us to rule out the alternative interpretation of an 18-month timelag.) Convection in the Labrador Sea takes place in the western central region⁴. This is about 700 km from where the convection signal was observed in the Irminger Sea some 6 months later, yielding a spreading speed of 4.5 cm s^{-1} . For processes removing newly ventilated waters from convected regions^{11,12} the coupling between the Labrador Sea and the Irminger Sea is thus intimate, rapid and efficient.

Adopting the circulation scheme proposed in refs 2 and 3, the LSW in the Iceland basin should be of more recent origin than that further east. All our records show the fresher LSW at 26–27° W and a significant erosion of the core salinity eastwards of that area. This reflects the length of the path from the source. Thus we assume that the LSW observed in locations south of the Rockall trough in December 1994 shows the arrival of the 1988 LSW cascade, because in 1991 and 1992 no significant property changes were detectable. In contrast, we observed significant changes ($\Delta\theta = -0.068^{\circ}\text{C}$ from 1991 to 1992 in the Iceland basin in those years where the new LSW signal had arrived earlier. We conclude that the new LSW took 4–5.5 years to propagate from its source region to the eastern boundary. With an assumed length of the pathway of 2,500 km, this is equivalent to a mean speed of about $1.5\text{--}2\text{ cm s}^{-1}$, at least 3 times faster than assumed earlier^{7,8,10}. Owing to the lack of data in the Labrador Sea in 1989, however, there is an uncertainty of one year

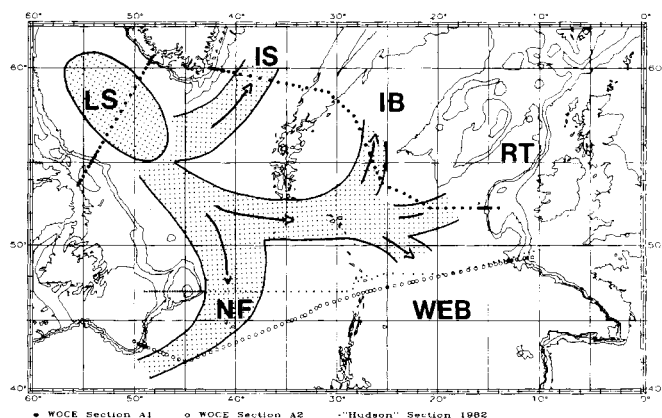


Figure 1 Main topography and schematics of primary pathways of the Labrador Sea Water^{2,3} and location of the WOCE lines A1/West (Labrador–Greenland), A1/East (Greenland–Ireland) and A2 (English Channel–Grand Banks). LS, Labrador Sea; IS, Irminger Sea; IB, Iceland basin; RT, Rockall trough; WEB, West European basin; NF, Newfoundland basin. The data along the hydrographic sections were acquired on the following cruises. A1/West: CSS 'Dawson' No. 90012/1, July 1990; CSS 'Hudson' No. 92014/1, June 1992 (not complete); CSS 'Hudson' No. 93019/1, June 1993; CSS 'Hudson' No. 94008/1, May/June 1994; RV 'Meteor' No. 30/3, Nov. 1994 (not complete, with CFC); CSS 'Hudson' No. 95011/1, June 1995. A1/East: RV 'Meteor' No. 18, September 1991 (with CFC); RV 'Valdivia' No. 129, Sept. 1992; RV 'Meteor' No. 30/3, November/December 1994 (with CFC); RV 'Valdivia' No. 152, June 1995. A2: CSS 'Hudson' No. 82001/2, April 1982; RV 'Gauss' No. 226/2, July 1993; RV 'Meteor' No. 30/2, October/November 1994; RV 'Gauss' No. 276/2, May/June 1996.

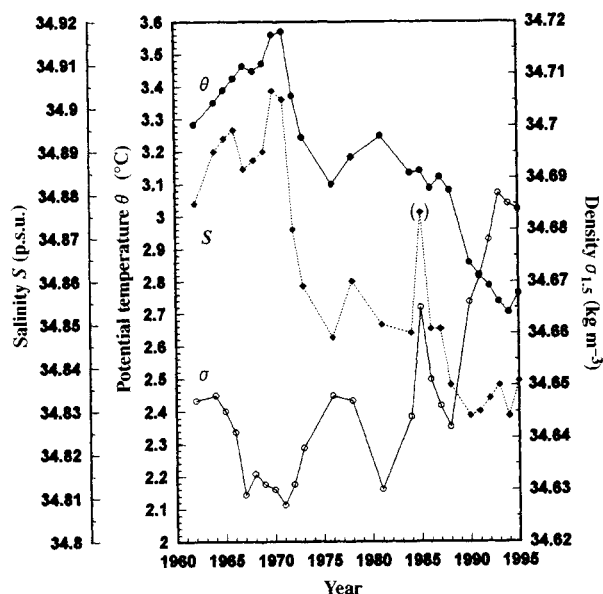


Figure 2 Time series of potential temperature (θ), salinity (S) and potential density anomaly (σ_{15}) of LSW from the central Labrador Sea (at the position of Ocean Weather Station Bravo) between 1962 and 1995 when data are available. Data are averaged from 1,000 to 1,500 dbar. The 1985 salinity value is questionable because it was sampled between the edge of the LSW and the higher-salinity boundary water.

Table 1 Changes of mean values of LSW core properties

Longitude	Δp (dbar)			$\Delta \theta$ ($^{\circ}\text{C}$)			ΔS			$\Delta \sigma_{\theta}$ (kg m^{-3})		
Section	A	B	C	A	B	C	A	B	C	A	B	C
A1/E												
>33°W	128	122	-15	-0.092	-0.140	-0.050	-0.002	-0.002	-0.006	0.007	0.011	0.0
<28-22°W	47	61	83	-0.068	-0.038	-0.082	0.0	0.0	-0.002	0.007	0.004	0.006
<22°W	103	112	-56	-0.003	-0.096	-0.010	0.002	0.002	-0.004	0.002	0.010	-0.002
Section A2												
>32°W	641	19	58	-0.450	-0.57	-0.103	-0.002	-0.001	-0.009	0.043	0.005	0.003
<32°W	27	125	38	-0.154	-0.096	-0.042	-0.026	-0.006	0.004	-0.006	0.005	0.007

These changes (of hydrographic properties of the intermediate θ/S -minimum of the LSW) are for the Irminger basin (>33°W), Iceland basin (28–22°W) and Rockall trough entrance (<22°W) along section A1/East, and for the Newfoundland basin (>32°W) and the West European basin (<32°W) along section A2. The columns A–F correspond to differences between two sets of observations, defined as follows: A1/East: A = 'Valdivia' 9/1992 – 'Meteor' 9/1991 (Δt = 12 months), B = 'Meteor' 12/1994 – 'Valdivia' 9/1992 (Δt = 27 months), C = 'Valdivia' 6/1995 – 'Meteor' 12/1994 (Δt = 6 months), A2: D = 'Gauss' 7/1993 – 'Hudson' 4/1982 (Δt = 135 months), E = 'Meteor' 10/1994 – 'Gauss' 7/1993 (Δt = 15 months), F = 'Gauss' 5/1996 – 'Meteor' 10/1994 (Δt = 18 months). Symbols: p , pressure (depth in ocean is measured in pressure units rather than in metres; a depth change of 100 dbar is equivalent to 100 m), θ , potential temperature; the temperature of a sample brought adiabatically to the surface, S , salinity, σ_{θ} , potential density anomaly, referenced to sea surface.

for the linkage of the 1988 LSW cascade between the remote and the source region.

Spreading at the sub-polar gyre's southern boundary

The above observations are supported by results from our southern section (WOCE Section A2), which we supplemented by the 1982 'Hudson' section as a reference for a period of former LSW production (see Fig. 1). Along the entire section we find a dramatic cooling of LSW in 1993 compared with 1982 (Fig. 4c), with the most prominent anomalies west of the MAR amounting to -0.45°C (-0.15°C east of the MAR). Associated with cooling we find a densification of 0.043 kg m^{-3} and a deepening of more than 600 dbar for the western basin (Table 1). Salinity remains almost unchanged west of the MAR but was found to be significantly

fresher (by about -0.03 p.s.u.) in the eastern basin. Similar to the northern section, the cooling continues, indicating the passage of the 1988 LSW cascade here as well. Between 1993 and 1996 the temperature decreased in the west by another -0.16°C and in the east by about -0.14°C : the newly formed LSW has already invaded the central West European basin and the dramatic ventilation of the LSW layer is currently in progress.

The attempt to assign the θ/S core values observed in 1993 and 1996 in the Newfoundland basin to an equivalent counterpart in the Labrador Sea, however, does not lead to a similar clear correlation as in the case for the Irminger Sea (Fig. 5b). This may be due to the more effective mixing along the longer pathway. In 1993 we observed a very patchy core layer which suggests the advection of LSW in terms of smaller-scale, intrusion-like lenses (that is, spatially separated small volumes of LSW). These are more susceptible to transformation by mixing than a large-scale uniform layer¹³.

In 1993 we also observed isolated LSW lenses in a narrow band close to the continental slope of the Grand Banks, which are fresher and some 600 m shallower than the deep LSW cores further east. This offset cannot be assigned to an equivalent signal in the Labrador Sea (Fig. 5b). Following ref. 13, we suggest that these three isolated θ/S core values are part of the non-classical LSW, termed 'upper LSW' and produced as sub-mesoscale lenses within the Labrador Current regime at the western border of the Labrador Sea gyre¹¹. If the offsets in density of the classical LSW in Fig. 5b are compared, the values from 1992 and 1994/95 from the central Labrador Sea correspond well to those from 1993 and 1996 from the Newfoundland basin. This implies that classical LSW has arrived at the Tail of the Grand Banks probably within one year. This high advection rate is only possible if the Deep Western Boundary Current is involved in entraining LSW¹¹.

Chlorofluorocarbon tracers

The estimated travel times of recently renewed LSW for the north-eastward and eastward export paths are consistent with independent estimates derived from CFC-11 measurements along WOCE Section A1 (Fig. 1) in 1991 and 1994. During the past decades the atmosphere and the upper ocean have been increasingly tagged with CFCs previously not existing in the environment. It is evident that the temporal change of deep convection activity in the Labrador Sea significantly modifies the CFC signal of LSW.

In 1991 and 1994 CFC concentrations were substantially lower east of the Reykjanes ridge (31°W) than in the Irminger Sea (Fig. 6a). In both the Irminger Sea and the northeast Atlantic, the CFC concentrations of LSW increased from 1991 to 1994, with the highest differences (0.8 pmol kg^{-1}) observed in the Irminger Sea. In 1994, CFC concentrations of LSW west of 22°W exceeded 2.5 pmol kg^{-1} with a maximum value of 2.8 pmol kg^{-1} near 26°W. The spreading LSW core is surrounded by water masses

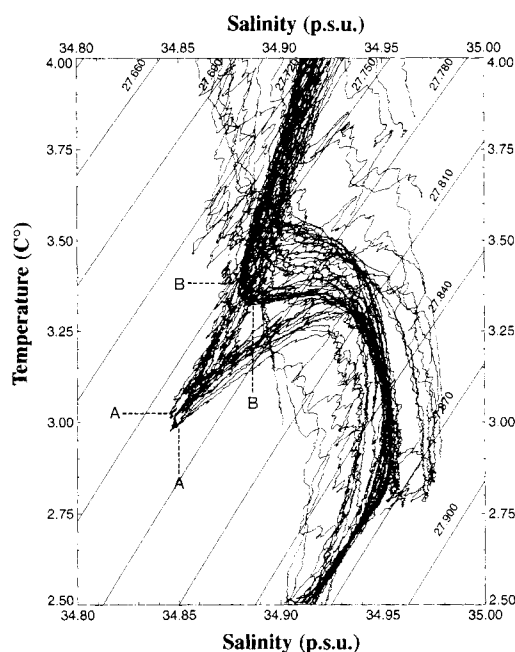


Figure 3 Temperature versus salinity (T/S diagram) along section A1/E compiled from all data of cruise 'Valdivia' 129 (9/1992) for the intermediate and deep subpolar North Atlantic. The well defined intermediate T/S minima mark the Labrador Sea Water (LSW): **a**, the colder and fresher group of minima at about $T = 3.0^{\circ}\text{C}$ and $S = 34.85 \text{ p.s.u.}$ characterizes the LSW core in the Irminger Sea; **b**, the warmer and saltier group of minima at about $T = 3.35^{\circ}\text{C}$ and $S = 34.88$ characterizes the LSW core in areas east of the Mid-Atlantic Ridge. This change of LSW core characteristics is due to mixing with surrounding water masses along the spreading path.

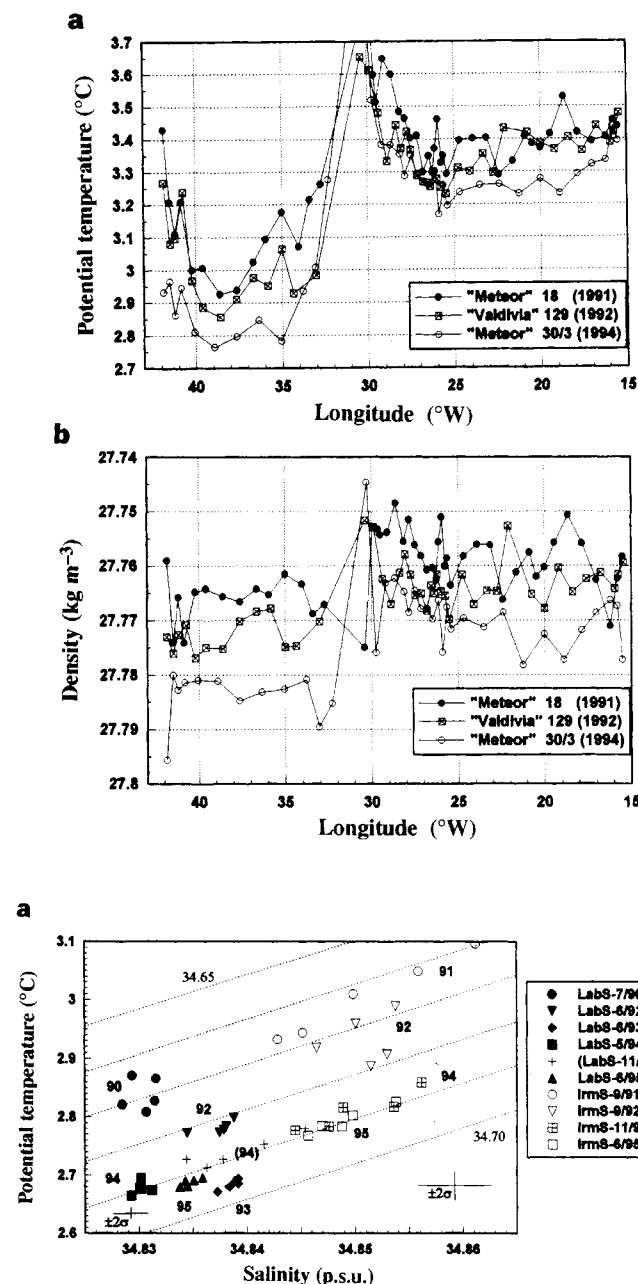


Figure 5 θ/S diagram of LSW cores for selected areas. **a**, Data collected between 1990 and 1996 in the central parts of the Labrador and Irminger seas (except for LabS-11/94 with values only from the northern part of section A1/W). Potential density anomaly (σ_{θ}) referenced to 1,500 dbar. Each value is calculated as the mean of a 200-dbar-thick layer centred at the θ/S peak of the LSW core. The mean 2 standard deviation for Labrador Sea and Irminger Sea core values are shown at

with lower CFC values, and its CFC concentration therefore decreases with mixing. Thus, the LSW observed in the northeast Atlantic in 1994 must have started in the Labrador Sea with concentrations higher than 2.8 pmol kg^{-1} , giving evidence that the LSW observed in 1994 in the northeast Atlantic represents LSW of vintages 1986 or younger (Fig. 6a). Timescales of 18–19 years (refs 7, 10) are not compatible with the 1994 observations in the northeast Atlantic, because in 1975 even the surface CFC concentrations in the Labrador Sea did not exceed 2.6 pmol kg^{-1} , that is, they were lower than observed 19 years later in the LSW in the northeast Atlantic. The CFC levels in 1991 in the northeast Atlantic (2.1 pmol kg^{-1}) were in the range of the pre-1986 values in the Labrador Sea⁵, so we cannot distinguish between timescales of 6

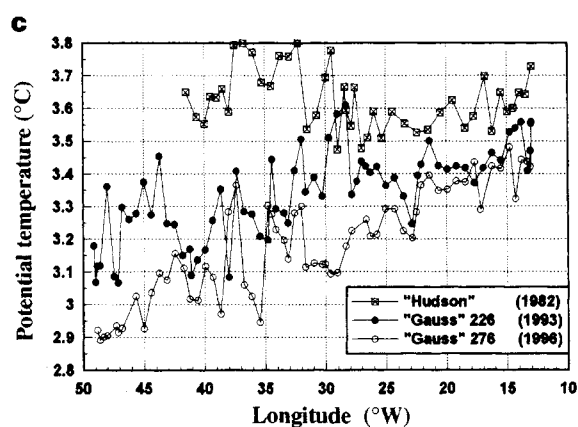
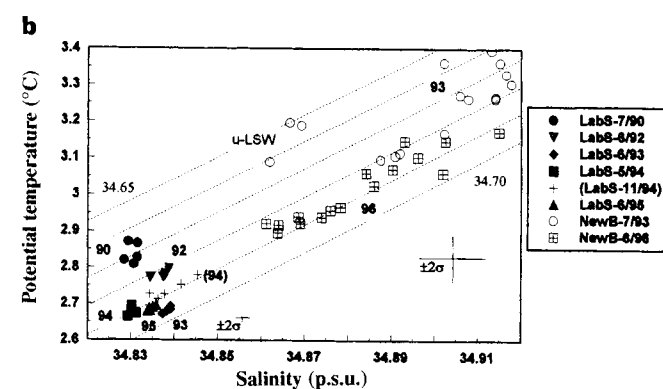


Figure 4 Change of LSW core properties along trans-ocean sections A1 and A2 (for clarity not all years are shown). **a**, Potential temperature at LSW salinity minimum along section A1/E in 1991, 1992 and 1994. **b**, Same as **a** but for potential density anomaly (σ_{θ}). **c**, Same as **a** but along section A2 for 1993 and 1996, and with data from CSS 'Hudson' from 1982 for comparison.



the left and right bottom respectively. From each cruise the five most central profiles were used. **b**, Same as **a** but for Labrador Sea and Newfoundland basin between 40°W and 49°W . The three isolated fresh and warm values marked with 'u-LSW' were observed in the Deep Western Boundary Current regime in 1993 and are suggested to be part of the 'upper LSW'.

years or 16 years. Surface CFC concentrations of 2.1 pmol kg^{-1} were present in the Labrador Sea in 1974, and so the propagation time was definitely faster than 16 years.

In 1994, CFC-11 concentrations in LSW observed in the Irminger Sea were only slightly lower than observed in the Labrador Sea at the same time (Fig. 6b). We know that intense deep convection occurred in spring 1994. Thus we conclude that the LSW observed in November 1994 in the Irminger Sea left the Labrador Sea in spring 1994. There were no measurements in 1991 in the Labrador Sea. The data from summer 1990¹⁴, however, showed CFC-11 concentrations which were about 0.2 pmol kg^{-1} lower in the temperature range $2.8\text{--}3.2^{\circ}\text{C}$ than found in the Irminger Sea in September 1991, so that the LSW in the Irminger Sea was probably

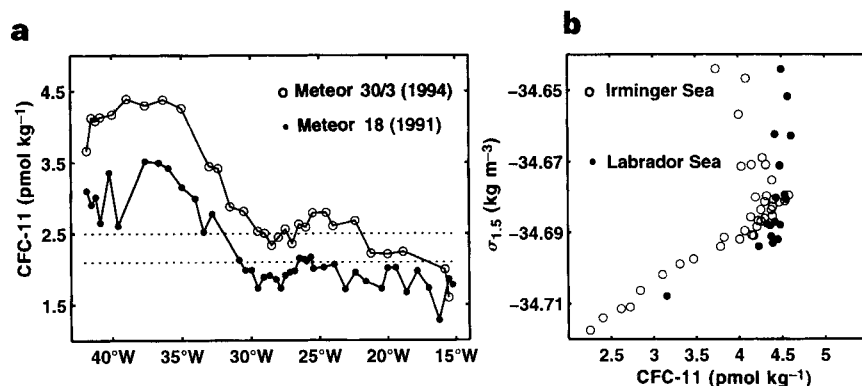


Figure 6 a, CFC-11 concentration of the LSW core, defined at the salinity minimum along the A1/E section in September 1991 and in November/December 1994. The dotted lines enclosing 2.1–2.5 pmol kg⁻¹ represents the LSW concentrations in the Labrador Sea before 1986⁶. The CFC levels increased after 1986 owing to the onset of deep convection. The effect of deep convection is to mix vertically the CFC profile from the surface to the convection depth¹⁰, thereby mixing the high CFC surface water with the LSW. In 1986, two types of LSW were observed; one

type was newly formed by convection with CFC concentrations of 3.1 pmol kg⁻¹ and the other represented older LSW with concentrations of 2.0–2.5 pmol kg⁻¹ at the potential density level $\sigma_{1.5} = 34.66 \text{ kg m}^{-3}$ (ref. 5). Owing to the absence of deep convection in 1977–85, the CFC-11 levels of LSW during that time period were 2.1–2.5 pmol kg⁻¹ or less. **b**, CFC-11 concentration versus potential density anomaly $\sigma_{1.5}$ in the density range of LSW in the Labrador Sea and the central Irminger Sea, November 1994.

a product of convection in spring 1991.

Discussion

Our *T/S* and CFC data independently show that LSW formed by convection since 1988 has taken about 4 to 5.5 years (mean speed 1.5–2 cm s⁻¹) to reach the Rockall trough area. This spreading rate is, however, incompatible with an earlier estimate of 18–19 years, which was based on a salinity decrease of -0.09 p.s.u. observed in the Rockall trough in 1990 (refs 7, 10). We will now argue that the discrepancy is not due to a recent change in spreading rates. Rather, we suggest that the earlier estimate is in error, because the decrease in Rockall trough salinity does not represent LSW freshening in 1972.

First, the freshening in the Rockall trough^{7,10} was larger than the entire salinity decrease observed at Ocean Weather Station *Bravo* in the central Labrador Sea since 1972 (Fig. 2). Moreover, the freshening is quite abrupt despite an assumed spreading time of 18–19 years. Second, the *T* and *S* variabilities of LSW in the formation area are eroded by a factor of 5 on their pathway into the northeastern Atlantic¹². This makes it unlikely that the freshening signal in the Rockall trough could be traced back into the Labrador Sea. The same argument makes the more pronounced cooling of the LSW (Fig. 2) a much better and much simpler spreading tracer than salinity. Direct velocity measurements are sparse in our observation area, but an annual mean eastward speed of 5 cm s⁻¹ for LSW in the central Labrador Sea in 1987¹⁵ is consistent with our findings. Third, the CFC observations in 1991 and 1994 are not compatible with the longer spreading rate estimate^{7,10}.

Model calculations¹¹ show that under extreme winter forcing, such as experienced in the Labrador Sea since the late 1980s, LSW might also be formed south of the classical formation area, that is, outside the western Labrador Sea gyre. The North Atlantic Current could then quickly export this newly formed LSW into the northeast Atlantic. In this case, LSW spreading would be faster than at times of weak winter forcing. Unfortunately, we have no hydrographic data to determine whether LSW was formed at the southern fringe of the Labrador Sea in recent years, but our data from the central Labrador Sea and from the northeast Atlantic suggest that the LSW vintages of 1991 and younger have not yet reached the eastern far-field of the sub-polar gyre of the North Atlantic. Any additional LSW formation area further south results in an even shorter path for the LSW spreading into the northeast Atlantic since 1989/90. Thus, shorter timescale estimates might be found in the future. Newly formed LSW naturally leaks also into the regime of the Subtropical Gyre of

the North Atlantic via the Deep Western Boundary Current system¹¹. Recent observations by M. S. McCartney (personal communication) suggest that LSW with characteristics similar to those we report here has arrived off Abaco, Bahamas.

We conclude that new modes of LSW affect the regions outside the Labrador Sea in the following sequence: (1) Irminger Sea after 6 months; (2) Newfoundland basin after about 1 year; (3) Iceland basin after 2–3.5 years; (4) western part of the West European basin; and (5) Rockall abyssal plain after 4–5.5 years. We hope that the new data, which have allowed us for the first time to quantify spreading rates for LSW signals by comparing time series of LSW properties at the source and at several remote locations, will be complemented by continued observations. This should improve our understanding of the mechanisms that transport newly ventilated waters into the ocean's interior, which strongly affect the low-frequency variability of its thermohaline circulation. □

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Disruption of orientation tuning in visual cortex by artificially correlated neuronal activity

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In the primary visual cortex, the development of orientation selectivity is influenced by patterns of neural activity. The introduction of artificially correlated activity into the visual pathway (through synchronous activation of retinal ganglion cell axons in the optic nerve) substantially weakens the orientation selectivity of neurons in superficial and deep cortical layers. This is consistent with activity having an instructive role in shaping cortical neuron receptive field tuning properties.

Neural activity is critical for the normal development of the mammalian visual system. Although the role of activity in the formation of ocular dominance columns has been well established^{1,2}, its role in constructing higher-order receptive field properties, such as orientation and direction selectivity, is controversial. Orientation columns in visual cortex are present before or at the time of eye opening^{3,4}, but the majority of neurons are still weakly orientation-selective at this developmental stage^{4,5}. This suggests that visual experience is not required for the initial formation of orientation columns, but rather endogenous spontaneous activity within the visual pathway may drive this process.

The maturation of orientation selectivity requires neuronal activity. Silencing cortical activity or preventing visual experience blocks the normal development of orientation-selective responses⁵. Because electrical activity within the visual pathway is substantially reduced during these experiments, it has remained unclear whether natural physiological levels of activity simply permit this developmental event to occur, or whether patterns of activity have a deeper, instructive role. If, as suggested for ocular dominance column formation^{6,7}, patterns of neuronal activity instruct the development of orientation and direction selectivity, then the presence of competing patterns of activity within the visual pathway should disrupt this process.

To test directly whether patterns of neural activity are important for the development of orientation and direction tuning, we introduced artificially correlated activity into the developing visual pathway of ferrets through chronic electrical stimulation of the optic nerve. Periodic bursts of pulses, applied through a cuff electrode implanted around the optic nerve, synchronously activated retinal ganglion cell axons. Optic fibre activation began about 1 week before eye opening (before the appearance of orientation maps³ and mature orientation selectivity⁵), and continued for about 2 weeks. In stimulated animals the number of cells in primary visual cortex with clear orientation and direction selectivity was markedly reduced when compared to unstimulated controls; however, the overall pattern of orientation and direction domains, as assessed by optical imaging, was unaffected. These findings demonstrate that patterns of correlated afferent activity can shape receptive field tuning properties, and suggest that cortical maps are resilient to changes in activity patterns that significantly alter the response properties of individual neurons.

Generation of artificially correlated activity

Ferret kits ($n = 6$) were raised with nerve-cuff stimulating electrodes chronically implanted at postnatal age (P) 15–17 around the

optic nerve of one eye; the other eye was enucleated at the time of cuff implantation to prevent binocular interactions during the development of orientation selectivity (Fig. 1a, b). Control animals ($n = 8$) underwent identical surgical procedures as stimulated animals, including cuff implantation and enucleation, but the optic nerve was not electrically stimulated. Stimuli consisted of 1.8-s bursts of 30 Hz biphasic current pulses (300 μ s duration) repeated every 20 s. Animals were stimulated 24 h a day, beginning at P18–20 until the time of physiological recording at P41–43. To ensure that animals were not being subjected to any discomfort,

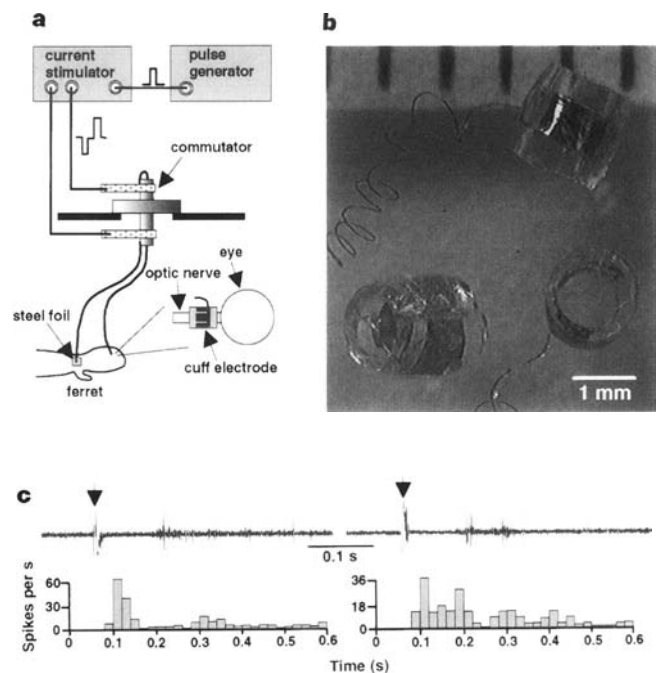


Figure 1 **a**, Diagram of the stimulation circuit (see Methods). **b**, Silicone nerve cuff electrodes of the split cylinder design with an inner circumferential band of platinum foil. **c**, Cortical spike discharges evoked by monopolar pulse stimulation applied to the optic nerve. Discharges are shown for two different sites from two different animals. Discharges in left trace were evoked by a current pulse of 1.0 mA, right trace by 0.9 mA. Upper traces show spike discharges evoked by a single pulse and the lower post-stimulus time histogram (PSTH) is an average of 20 trials. Arrows indicate the stimulus artefact. There was an 80–90 ms latency in the evoked cortical spikes following pulse application.

current levels were always set below the threshold that might elicit any behavioural responses during the stimulus bursts. Stimulus currents were increased daily from an initial level of 0.3–0.4 mA at P18–20 to 2.0 mA at the time of physiological recording (see Methods). Because bipolar optic nerve recordings revealed that the minimum current required for eliciting optic nerve responses was 0.8–0.9 mA, optic nerve fibres were not functionally activated by the cuff electrode until P27–29 when stimulus currents greater than this magnitude could be applied without discomfort to the animals (see Methods). Thus, effective visual system activation began at P27–29 in our stimulated test animals. This is before the age when orientation maps can first be detected using optical imaging³ and before the maturation of orientation tuning as assessed by single-unit recording⁵. Control and stimulated animals were allowed similar periods of visual experience for about 7 to 10 days following natural eye opening at P33–35.

Disruption of orientation selectivity

To assess the ability of optic nerve stimulation to activate cortical neurons, we recorded spike activity of neurons in primary visual cortex evoked by single monopolar pulses applied to the cuff at the end of the stimulation period. Single pulses (0.9 to 2.0 mA, the same as used during the chronic stimulation) applied to the cuff evoked bursts of cortical cell discharge at 78% of the recording sites (Fig. 1c) ($n = 176$ sites in 4 animals), indicating that cuff stimulation activated neurons at multiple levels of the visual pathway. The threshold for eliciting cortical spike discharge was 0.9–1.1 mA, which was slightly above the threshold for optic nerve activation. In two animals, spike discharges could not be evoked at any cortical sites because of a broken wire to the cuff electrode. These animals were not studied further.

The orientation selectivity of neurons in primary visual cortex

was assessed by recording extracellular spike activity from single neurons in response to high-contrast bars drifting at 18 different orientations. To prevent sampling bias, we recorded at cortical sites spaced at 150- μ m intervals along semi-tangential penetrations, at about a 30° angle to the cortical surface. At each site spikes of one to three neurons could be readily discriminated from multi-unit activity using waveform template analysis. Discriminated cells at a single site had similar peak orientation preferences and degrees of selectivity. For each isolated unit, an orientation selectivity index (OSI) was calculated using Fourier analysis, by extracting the amplitude of the second harmonic from the orientation tuning curve, normalized by dividing by the mean firing rate of the cell during stimulus presentation⁸ (Fig. 2a, d). Small OSI values indicate weak selectivity whereas large values indicate high selectivity.

In the four animals from which visual cortical spike discharges were evoked by cuff stimulation, orientation selectivity was dramatically reduced when compared to control animals. We recorded from a total of 334 neurons at 176 sites in stimulated animals and 271 neurons from 162 sites in controls. In stimulated animals only 17% of cells had robust orientation selectivity ($OSI \geq 8$) whereas in control animals 60% of cells had an $OSI \geq 8$ (Fig. 2a, d). These differences were highly significant ($P < 0.001$, Mann–Whitney T -test). Weakly tuned cells with an OSI of between 2 and 4 were most prevalent in stimulated animals whereas strongly tuned cells with an OSI of between 10 and 12 were most prevalent in control animals (Fig. 2a). The OSI distributions of control animals were consistent with previous reports that the majority of neurons in adult ferret primary visual cortex are well tuned for orientation⁵ and with previous findings that monocular enucleation does not affect development of normal orientation tuning⁹. In contrast to control animals, the distribution of OSIs in stimulated animals closely resembled that from immature ferrets before eye opening⁵. We

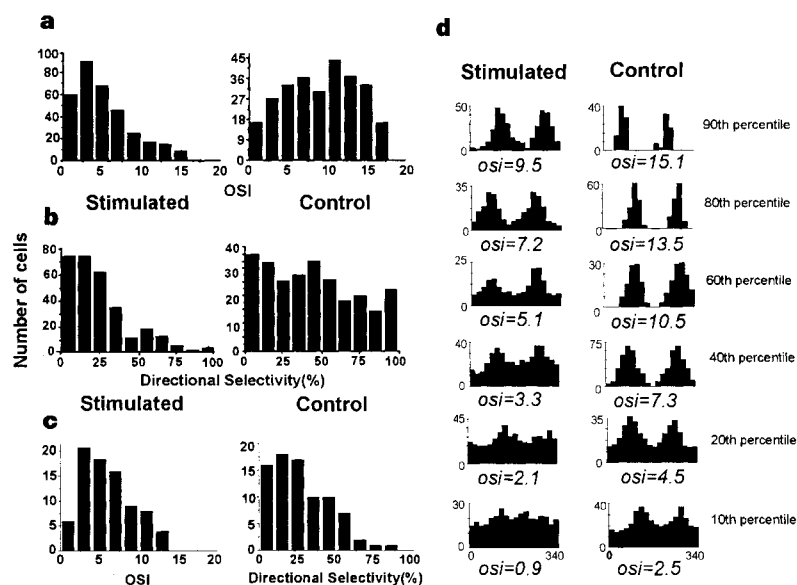


Figure 2 Stimulated animals show reduced orientation selectivity when compared to unstimulated controls. **a**, Histograms of orientation selectivity index (OSI) distributions for neurons in stimulated test ($n = 334$ neurons) and control ($n = 271$ neurons) animals. This set of test animals were stimulated from age P17–18 with daily increases in current amplitude. Small OSI values represent weak orientation selectivity. The OSI distribution for stimulated animals is significantly shifted towards low values when compared to controls. **b**, Histograms of the directional selectivity index for all stimulated and control animals. Stimulated animals have substantially lower directional selectivity indexes as compared to controls. **c**, Histograms of orientation and direction selectivity for the test animal in which stimulation was started at P28 ($n = 135$ neurons). These histo-

grams are similar to those obtained from stimulated animals shown in **a** and **b**. **d**, Orientation tuning curves of representative neurons are shown according to their percentile ranking within each stimulated or control group. In each graph the y-axis indicates the average peak cell firing rate, the x-axis indicates the 18 different directions of motion of the bar stimulus (0° to 340°) where the direction of motion is perpendicular to bar orientation. Under each tuning curve is the corresponding calculated OSI. A substantially larger percentage of cells in the control group have strong orientation selectivity as compared to the stimulated group. For example, in control animals a cell in the 40th percentile has robust orientation selectivity with an OSI of 7.3, whereas in stimulated animals a cell in the 40th percentile cell has substantially weaker orientation selectivity with an OSI of 3.3.

also observed a reduction in the degree of direction selectivity between stimulated and control animals. In stimulated animals only 17% of cells had directional biases greater than 2 to 1, whereas in control animals 50% of cells had directional biases greater than 2 to 1 (Fig. 2b). These differences were also highly significant ($P < 0.01$, Mann-Whitney T -test).

In a second set of animals, cuffs were implanted at P15–17 but no stimulation was applied until P28. In these animals, we directly confirmed that optic nerve activation beginning at P28 until the time of recording at P41–43 was sufficient to reduce orientation selectivity. Stimulation currents of 1.8 mA, which were above the saturation level for optic fibre activation (see Methods), could be directly applied at P28 in these animals without eliciting any behavioural response ($n = 3$). Current intensities were increased daily to a maximum of 2.3 mA. In two animals, cuff stimulation did not evoke spike activity at any cortical sites during recording sessions at the end of the stimulation period because a broken wire to the cuff electrode. In the remaining animal, robust spike activity was evoked from 73% of cortical recording sites. The degree of orientation and direction tuning in this animal (Fig. 2c) was significantly reduced when compared to unstimulated controls ($P < 0.001$ (orientation), $P < 0.01$ (direction), Mann-Whitney T -test) and was similar to that found in the previous set of stimulated animals (Fig. 2a, b).

The reduction of selectivity in stimulated animals did not result from sluggish or unresponsive neurons, because neurons both in stimulated and in control animals had similar peak firing rates during visual stimulation (10 to 90 spikes per s). Moreover, there was no correlation between the orientation selectivity of cells and their peak firing rate either in stimulated or in control animals (stimulated, $r = 0.11$; control, $r = 0.09$; Pearson product-moment

correlation). These findings are similar to those observed in normal ferrets of the same age⁵. In addition, cortical sites strongly activated by the stimulus pulses had weaker orientation selectivity than sites that could not be robustly driven. There was a significant negative correlation between the ability of cuff stimulus pulses to evoke cortical spike discharges and the OSI at a given cortical recording site ($r = -0.42$, Pearson product-moment correlation). We did not find any correlation between stimulus pulse activation and laminar position: cells located at all electrode depths were equally likely to be activated by stimulus pulses.

Laminar analysis of orientation tuning

Along single electrode penetrations in stimulated animals, we observed clusters of neighbouring sites with similar OSIs and continuous shifts in OSI between adjacent sites. For example, in Fig. 3a there is clustering of sites with very weak orientation selectivity at locations 3–5. There are also continuous shifts in orientation selectivity throughout the penetration, most noticeable across locations 17–21 in which there is a gradual increase in orientation selectivity as the electrode progresses through the lower cortical layers. We could not find any statistically significant pattern to the shifts in OSI along single penetrations, and no correlation between the OSI and cortical depth. Cells at almost all cortical depths had weak orientation tuning with OSIs between 3.5–5.5 (mean OSI = 4.6) (Fig. 3c). In unstimulated control animals, orientation tuning also shifted continuously throughout the penetration. However, cells located in upper and lower cortical layers had significantly sharper orientation tuning (mean OSI = 11.8) than cells located within intermediate layers (mean OSI = 5.3) (Fig. 3b, d), consistent with previous findings⁵. These results indicate that cells within intermediate layers in both stimulated and control

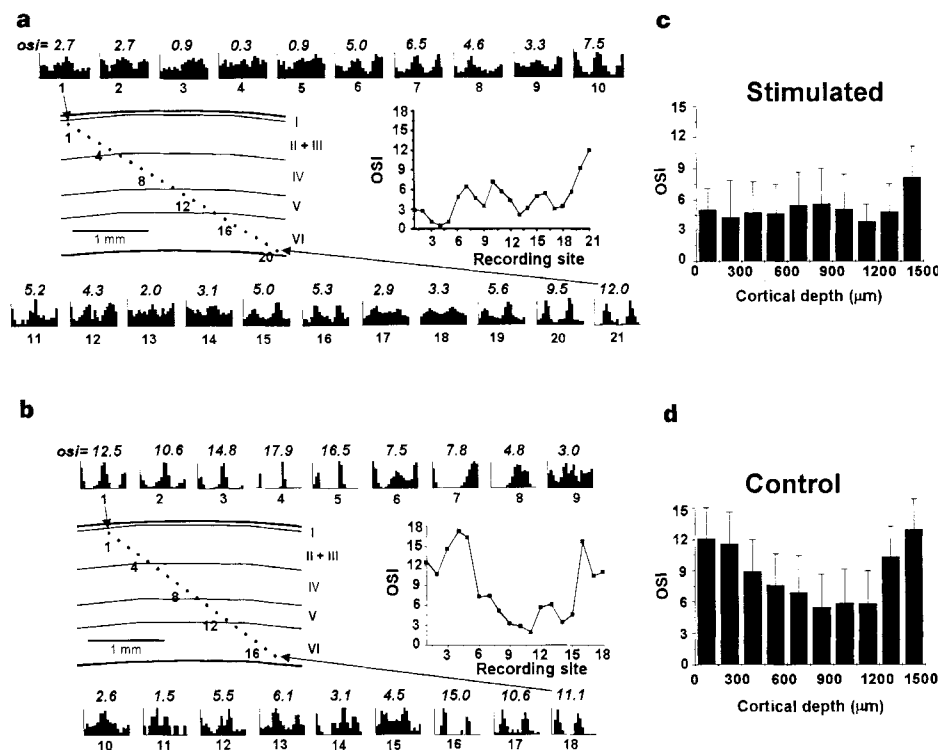


Figure 3 Orientation tuning curves and corresponding OSI values for all sites recorded along a single electrode penetration in a stimulated (**a**) and control (**b**) animal. The locations of recording sites are shown in the central inset diagram. The linear distance between recording sites was typically 150 μm. The inset graph in **a** and **b** plots the shift in OSI with respect to the recording sites. **a**, Stimulated animal. OSI values are typically below 6 throughout the penetration except at the deepest sites. **b**, Control animal. Sites located in superficial or deep

layers have stronger orientation selectivity, with OSIs from 10 to 17, than sites within intermediate layers, with OSIs from 1 to 7. **c, d**, Quantification of OSI versus depth for all penetrations in stimulated (**c**) and control (**d**) animals. The orientation selectivity in stimulated animals remains relatively weak across all cortical layers except for a slight increase at the deepest sites. In contrast, the orientation selectivity in control animals is highest in superficial and deep layers and lowest in intermediate layers.

animals had similarly weak orientation selectivity; however, in control animals the orientation tuning of cells in upper and lower layers was significantly sharper than in stimulated animals. This suggests that chronic stimulation preferentially disrupts intrinsic cortical circuits underlying the sharpening of orientation tuning within superficial and deep cortical layers.

ON/OFF LGN responses

To ascertain whether the site of the disruption was cortical, we recorded from the lateral geniculate nucleus (LGN) ($n = 16$ cells). If chronic stimulation produced alterations in the ON/OFF responses of LGN neurons, this could weaken cortical orientation selectivity, possibly by affecting the spatial arrangement of ON/OFF subregions within simple cells¹⁰. Activity-dependent modifications of ON/OFF responses have been reported¹¹, and in ferrets activity contributes to segregation of ON/OFF layers within the LGN between P7 and P21¹². Activation of optic nerve fibres by the cuff electrode, which began at P27–29, occurred well after this segregation process was completed. All isolated units recorded in the LGN exhibited either purely ON or OFF responses; no units with mixed responses were encountered (Fig. 4). Furthermore, we observed a consistent shift from one response type to another with changes in electrode depth, indicating that the laminar segregation of ON and OFF units was intact in stimulated animals. The finding of normal ON/OFF LGN units also rules out the possibility that ON/OFF responses in retinal ganglion cells were disrupted by antidromic activation of their axons in the optic nerve.

Organization of cortical maps

Although orientation selectivity in stimulated animals was significantly weaker than in control animals, the overall organization of orientation columns across primary visual cortex was not altered. Differential optical imaging maps of cortical activation obtained from orthogonal grating stimuli revealed a normal size, spacing and pattern of orientation domains (Fig. 5a, b); however, the magnitude of the signal in these maps was on average 1.6 times weaker than in control animals (Fig. 5e–g). This would be expected from the extracellular recordings that demonstrated reduced orientation selectivity of individual cells. Vector summation of the differential images into angle maps also revealed normal and continuous shifts of orientation preference (Fig. 5c). This was confirmed by extracellular recording which revealed that the peak orientation preference of cells typically shifted continuously along the electrode track. The average cycle period of orientation changes was $910 \pm 50 \mu\text{m}$ using autocorrelation analysis on angle maps, which is equivalent to previous reports of $890 \pm 29 \mu\text{m}$ in adult ferret¹³. Unstimulated control animals also had a similar cycle period of

$880 \pm 37 \mu\text{m}$. Optical imaging also revealed a clustering of directional selective responses into spatially defined direction domains, as previously reported in normal ferrets¹³ (Fig. 5d).

Discussion

If chronic stimulation interferes with the development of orientation and direction selectivity by disrupting naturally occurring patterns of neural activity, what are the sources of this activity? During the first postnatal month, before eye opening, patterned spontaneous activity is present in the ferret retina as waves of correlated ganglion cell firing^{14,15}. Correlations in spontaneous firing also occur preferentially between neighbouring ON ganglion cells or OFF ganglion cells in the mature retina¹⁶ and within retinal waves of immature animals¹⁷. Intrinsic synchronized neuronal oscillations have also been observed in the developing LGN¹⁸. Additionally, local correlations in neural firing are produced by visually driven activity due to the inherent statistical structure of natural scenes¹⁹. Because optic nerve stimulation began as retinal waves subsided, stimulation probably did not compete with patterned visual system activity induced by these waves. Therefore, it is more likely that stimulation primarily competed with the effects of post-retinal wave spontaneous and visually driven activity on the development of orientation and direction selectivity. For example, coactivation of both ON and OFF ganglion cell axons by chronic stimulation would produce abnormally correlated activity between ON and OFF inputs to simple cells, which could disrupt simple cell receptive field development^{20,21}.

There are a number of possible reasons why stimulation did not affect the overall structure of orientation and direction preference maps, while markedly disrupting the selectivity of individual cells. Because thalamic axons begin to arrive in cortical layer IV at P10²², patterning of synaptic connections may already establish an initial map structure before stimulation of the optic nerve begins at P27–29. Although stimulation of the optic nerve began 3 to 9 days before orientation preference maps can be first observed using optical imaging³, orientation map structure may be present that cannot be detected by this method at these early ages. Our stimulation protocol also activates the visual pathway for only 10% of the time; therefore, normal patterns of neural activity that are present during the remaining 90% of the time could potentially reduce the overall effects of stimulation. In addition, previous work has demonstrated the remarkable stability of cortical maps during the maturation period of orientation tuning. For example, organized orientation maps are only first observed in P32–35 animals³ at a time when the majority of neurons still have weak orientation selectivity⁵, followed by the sharpening of orientation selectivity during which the structure of the map remains unchanged³. Reverse suture experiments in cats also indicate that visually driven activity, which profoundly alters the response properties of visual cortical neurons, has little influence on the organization of cortical maps^{23,24}. Furthermore, computational models of orientation tuning development suggest that different mechanisms underlie the development of cortical maps and receptive fields: local intracortical connections determine the overall spatial organization of orientation preference maps, while spatio-temporal patterns of correlated afferent activity determine the receptive field substructure of orientation-selective simple cells^{20,21,25,26}. Taken together, these experimental and computational studies suggest that disruption of afferent activity patterns could modify the receptive field tuning properties of individual cells without altering the organization of cortical maps, as our results demonstrate.

It has been proposed that the growth and refinement of synaptic connections occurs by mechanisms in which synchronously firing inputs are stabilized onto common postsynaptic target neurons while nonsynchronous inputs are destabilized^{27,28}. In this way, the synchronous activation of neurons by chronic stimulation may be viewed as adding 'noise' to the information contained within

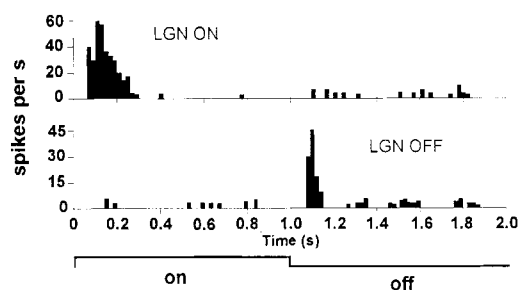


Figure 4 LGN cells in stimulated animals exhibit either purely ON or OFF responses. The upper post-stimulus time histogram (PSTH) shows a cell that responds to light on and the lower PSTH shows a cell that responds to light off. Each PSTH is the average of 15 trials. The timing of stimulus light on and off are shown at the bottom of the figure.

neuronal firing patterns, thereby reducing the amount of natural correlations present in ongoing activity. This could lead to the growth and stabilization of inappropriate synaptic connections between thalamic afferents and cortical neurons or within local cortical circuits, resulting in the reduced specificity of orientation- and direction-selective responses. □

Methods

Cuff implant surgery. All surgery was performed under sterile conditions. Ferrets (P15–17) were anaesthetized by freely breathing halothane in oxygen (2–3% induction, 0.5–1.5% maintenance). Facial muscles lying caudal to the orbit were retracted allowing access to the optic nerve beneath the eyeball. The

optic nerve was dissected free from surrounding connective tissue, and the cuff was placed loosely around the optic nerve to minimize damage to the nerve. The wire lead from the cuff was brought to the skin about 1 cm caudal to the eye. The facial muscles and overlying skin were sutured, and sterile gelfoam was used to fill any air gaps within the incision. An 8 mm² piece of stainless steel foil, 25 μ m thick (Goodfellow Corp., Berwyn, PA), was placed subcutaneously in the shoulder. Wire leads from the stainless steel foil and cuff were attached to an adjustable harness placed around the animal's chest. The other eye was removed at the time of cuff implantation by reaching behind the exposed eyeball with fine scissors and cutting the optic nerve. The orbit was packed with sterile gelfoam and the overlying skin sutured close. Following surgery, animals were raised in individual cages with 12-h light/dark cycles. They were hand fed Esbilac (PetAg Inc.) every 4 to 12 h depending upon age, and monitored for normal weight gain. All surgeries and stimulations were undertaken with the approval of the Duke University Institutional Animal Care and Use Committee. During the stimulation period, the resistance of the electrode was checked daily in order to assess whether the stimulation circuit was intact. However, this method could only determine whether a wire had broken outside the body, which was immediately fixed, but not whether it had broken inside the body. A broken wire inside the body could only be identified at the end of the experiment when cuff stimulation did not evoke cortical spike activity, and these animals were not included in our data.

Nerve cuff fabrication. The cuff electrode consisted of a single circumferential band of 25- μ m-thick platinum foil (Goodfellow Corp., Berwyn, PA) embedded within a silicone casing. Two types of silicone cuff casings were used, a split cylinder and a self-sizing spiral design. The spiral design was fabricated according to methods described previously²⁹. The split cylinder design was fabricated by wrapping the platinum foil around a 20–21 g needle, and then coating the needle with a thin layer of Silastic elastomer (MDX4-421, Dow Corning) which was slit and peeled off together with the foil from the needle after curing. Cuffs had an internal diameter of about 0.8–0.9 mm which were slightly oversized for the nerve at the time of implantation. For both cuff types, the first 1 cm of lead from the cuff consisted of multistranded Teflon-insulated 110 μ m diameter stainless steel wire (A-M Systems, Everett, WA). This wire was coiled to allow its overall length to increase as the animal grew in size.

Stimulation circuit. A pulse generator (Master 8, AMPI, Jerusalem, Israel) triggered a constant current stimulator (WPI) which produced biphasic pulses (see Fig. 1a). Each pole of the stimulator was connected to insulated ends of a commutator placed above the animal's cage. The commutator allowed the animal to move freely around the cage while maintaining electrical contact with the stimulator. One wire was connected to an 8 mm² piece of stainless steel foil implanted subcutaneously in the shoulder, the second wire was connected to the cuff electrode implanted around the optic nerve just behind the eyeball within the orbit. The circuit was connected such that the initial negative going current pulse was applied to the optic nerve electrode.

Effectiveness of optic nerve stimulation. To test the ability of cuff electrode to stimulate optic nerve fibres, we measured the stimulus current threshold and saturation levels for evoking compound action potentials in the optic nerves in a separate set of three animals. These animals underwent identical cuff implant surgery at P15–17 as performed on stimulated test animals. The minimum current level for eliciting optic nerve responses was 0.8–0.9 mA and the responses saturated at 1.5–1.7 mA when tested 5, 6 and 10 days after implantation. Using these current levels as a guide we determined that in test animals stimulated from P18–20, the threshold current for optic fibre activation was reached at P25–26 and the saturation current was reached at P29–30 (stimulus currents applied to test animals: 0.3–0.4 mA/P18–20, 0.8–0.9 mA/P25–26, 1.5–1.6 mA/P29–30, 2.0 mA/P34–43). Thus, effective visual system activation began at about P27–29. Maximum stimulus currents of 2.0 mA were applied in order to minimize the risk of damage to the optic nerve.

Optic nerve recording. Ferrets (P23–27) were anaesthetized with a mixture of ketamine hydrochloride (40 mg kg⁻¹, intramuscular (i.m.)) and xylazine hydrochloride (2 mg kg⁻¹, i.m.). The skull overlying the frontal cortex was first removed, and the frontal cortex above the area where the optic nerves enter the cranium was removed by suction. A bipolar hook electrode made from two 50 μ m diameter tungsten wires was used to record compound action potentials from the exposed optic nerve.

Cortical electrophysiological and optical recording. See ref. 13 for more

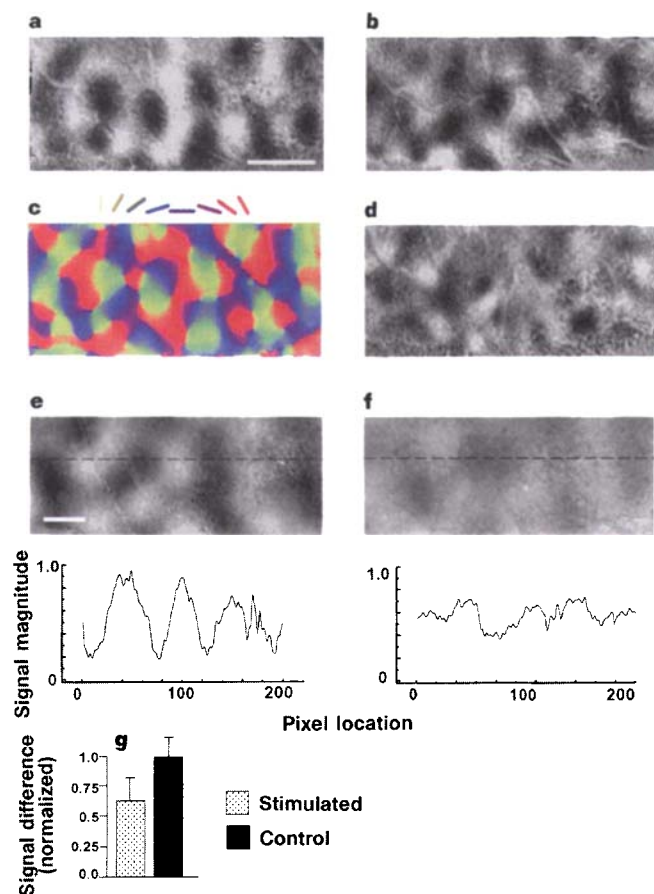


Figure 5 Differential optical images of orientation domains obtained from 90°/0° (vertical/horizontal) gratings in **a**, and 45°/135° gratings in **b** from one stimulated animal. Scale bar, 1 mm for **a–d**. The layout of orientation domains is similar to that seen in control animals. Minimum and maximum signal values were scaled to the full range of greyscale levels (also applies to **d**). **c**, Angle map of orientation preference from the same cortical region shown in **a, b**. The normal organization of orientation preference is preserved. **d**, Differential optical image of direction domains obtained from 0°/180° motion from the same stimulated animals shown in **a–c**. The normal organization of direction domains is also intact. **e**, Differential optical image obtained from 90°/0° gratings in a control animal. Scale bar, 0.5 mm for **e** and **f**. **f**, Differential optical images obtained from 90°/0° gratings in a stimulated animal. Images in **e** and **f** were scaled to the same signal range in order to compare the magnitude of the signal in both images. Under each image in **e** and **f**, signal intensity is plotted across the dashed lines shown in the images. There is a roughly 1.8-fold reduction in the signal obtained from the stimulated animal in comparison to that obtained from the control animal. **g**, Normalized signal magnitude difference in stimulated and control animals (see Methods). There is a 1.6 reduction of signal in stimulated animals as compared to control animals.

information about methods. Ferrets (P41–43) were anaesthetized with a mixture of ketamine hydrochloride (40 mg kg⁻¹ i.m.) and xylazine hydrochloride (2 mg kg⁻¹ i.m.). A hole (6 mm by 12 mm) was made in the cranium over area 17, the dura was removed, and a stainless steel chamber was mounted to the skull filled with saline and sealed with a coverglass. Ferrets were paralysed to prevent eye movements with pancuronium bromide (2–4 mg kg⁻¹ intraperitoneally (i.p.)) and artificially respiration with a mixture of 2:1 N₂O/O₂ supplemented with 0.5–1.0% halothane. Expired CO₂ was maintained at 4–5%. Pupils were dilated with 2% atropine. Corneas were protected from dehydration with zero-power contact lenses. Visual stimulation was provided monocularly through the contralateral eye at a distance of 30 cm. The stimulus for imaging orientation domains was a bar grating pattern (1.2 degree bar width, 6 degree bar spacing) that was drifted at 18 deg s⁻¹. Gratings were presented at 8 different orientations. The stimulus for imaging direction domains consisted of an array of randomly placed high-contrast bars (1.2 by 4.2 degrees) drifted at 18 deg s⁻¹. Typically, only one pair of directions were imaged for each animal. Images were acquired using an enhanced video acquisition system (Optical Imaging Inc., New York). To compare the signal magnitude of differential maps obtained from stimulated and control animals, we first made plots of the signal intensity across 50 to 100 randomly chosen rows of each image (see Fig. 5e). The values at peaks and troughs in all plots were separately averaged (peaks represent cortical activation from one orientation and troughs represent activation from the orthogonal orientation). Average peak and trough values for all maps within each stimulation or control group were averaged, and then subtracted from one another to produce the graph in Fig. 5g. This method measured the depth of modulation of the optical signal across the cortex.

Following optical recording, the coverglass on the steel chamber was removed, the saline drained and a sheet of 50-μm-thick silicone was laid over the exposed cortex. A hole was made in the silicone sheet and a tungsten recording electrode (14–18 Mohm, FHC, Brunswick, ME) was inserted through the hole just touching the cortex. Warm agar (2–4%) was applied over the silicone, which after hardening prevented cortical pulsations. Visual stimulation was provided by a single high-contrast bar (1.2 degree bar width, 40 degree length) presented at 9 different orientations and drifted at 18 deg s⁻¹. At each orientation, spike activity was recorded as the bar moved in one of two opposite directions across the centre of the cell's receptive field. For each of 18 different directions of motion, 4 to 8 trials were averaged. Spike activity was recorded and waveform discriminated using SPIKE2 (Cambridge Electronic Design, Cambridge, UK). For constructing orientation tuning curves, the peak discharge rate at each direction of motion was determined during a 1.0–1.5-period as the stimulus bar swept through the central region of the receptive field, and background spontaneous activity was subtracted. The depth and location of recording sites was assessed by first measuring the angle of the electrode to the cortical surface (30° to 40°), and then plotting points according to the total electrode distance travelled to each recording site. The linear distance between recording sites was typically 150 μm.

Orientation selectivity index calculation. The orientation selectivity index (OSI) was calculated as described previously⁸. Using Fourier analysis, the second harmonic component was extracted from the orientation tuning curve and normalized by dividing by the mean firing rate of the cell during stimulus presentation:

$$A = \sum_{i=1}^{18} (\text{OrienCurve})_i \times \sin(40^\circ \times i)$$

$$B = \sum_{i=1}^{18} (\text{OrienCurve})_i \times \sin(40^\circ \times i)$$

$$C = \sqrt{A^2 + B^2}$$

$$\text{OSI} = C/(\text{MeanFiring})$$

where 'OrienCurve' is the eighteen-element orientation tuning curve obtained from the spike data. The multiplier, 40, scales the sin and cos functions to produce two cycles spanning the eighteen sampled directions of motion, where the angle is measured in degrees.

Direction selectivity calculation. To calculate directional selectivity, we first found the peak orientation preference from the tuning curve. At this orientation, we calculated the firing rates for stimulus motion in opposite directions. The largest response was divided by the smaller response and expressed as a percentage. This method allowed directional selectivity to be calculated without being confounded by overall orientation selectivity, especially for cells with weak orientation selectivity.

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Transient optical emission from the error box of the γ -ray burst of 28 February 1997

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For almost a quarter of a century¹, the origin of γ -ray bursts—brief, energetic bursts of high-energy photons—has remained unknown. The detection of a counterpart at another wavelength has long been thought to be a key to understanding the nature of these bursts (see, for example, ref. 2), but intensive searches have not revealed such a counterpart. The distribution and properties of the bursts³ are explained naturally if they lie at cosmological distances (a few Gpc)⁴, but there is a countervailing view that they are relatively local objects⁵, perhaps distributed in a very large halo around our Galaxy. Here we report the detection of a transient and fading optical source in the error box associated with the burst GRB970228, less than 21 hours after the burst^{6,7}. The optical transient appears to be associated with a faint galaxy^{7,8}, suggesting that the burst occurred in that galaxy and thus that γ -ray bursts in general lie at cosmological distance.

GRB970228 was detected⁹ with the Gamma-ray Burst Monitor¹⁰ on board the Italian–Dutch BeppoSAX satellite¹¹ on 1997 February 28, UT 02 h 58 min 01 s. The event lasted ~ 80 s and reached peak fluxes of $\sim 4 \times 10^{-6}$, $\sim 6 \times 10^{-6}$ and $\sim 10^{-7}$ erg cm⁻² s⁻¹ in the 40–600 keV, 40–1,000 keV and 1.5–7.8 keV ranges, respectively^{9,12} (note that the peak flux of 0.23 Crab quoted in ref. 9 is in error). It occurred in the field of view of one of the BeppoSAX Wide Field Cameras (WFCs)¹³. The spectrum of the event is characteristic of classical γ -ray bursts (GRBs)¹². Its position (about halfway between α Tauri and γ Orionis) was determined with an accuracy of 3' (radius)⁷ at right ascension (RA) 05 h 01 min 57 s, declination (dec.) $+11^\circ 46.4'$. Application of the long-baseline timing technique¹⁴ to the GRB data obtained with the Ulysses spacecraft, and with the BeppoSAX and the Wind satellites, respectively, constrained this location to be within each of two parallel annuli, with half-widths^{15,16} of 31" (3 σ), and 30" (3 σ), respectively, which intersect the WFC error circle (Fig. 1).

Eight hours after the burst occurred, BeppoSAX was reoriented so that the GRB position could be observed with the LECS and MECS detectors^{17,18}. A weak X-ray source was then found¹⁹ at RA

05 h 01 min 44 s, dec. $+11^\circ 46.7'$ (error radius 50"), near the edge of the WFC error circle⁹ (Fig. 1). The 2–10 keV (MECS) flux of this source was 2.4×10^{-12} erg cm⁻² s⁻¹. The LECS instrument measured a 0.1–10 keV source flux of $(2.6 \pm 0.6) \times 10^{-12}$ erg cm⁻² s⁻¹. The source spectrum was consistent with a power-law model with photon index 2.7, reduced at low energy by a column density N_H of 5.6×10^{21} cm⁻². During an observation with the same instruments on March 3, UT 17 h 37 min this flux had decreased by a factor of 20 (ref. 19). With ASCA the X-ray source was detected²⁰ on 7 March at a 2–10 keV flux of $(0.8 \pm 0.2) \times 10^{-13}$ erg cm⁻² s⁻¹.

On February 28, UT 23 h 48 min, 20.8 hours after the GRB occurred, before we had any knowledge of the X-ray transient, we obtained a V-band and an I-band image (exposure times 300 s each) of the WFC error box with the Prime Focus Camera of the 4.2-m William Herschel Telescope (WHT) on La Palma²¹. The $1,024 \times 1,024$ pixel CCD frames (pixel size 24 μ m, corresponding to 0.421") cover a $7.2' \times 7.2'$ field, well matched to the size of the GRB error box. The limiting magnitudes of the images are $V = 23.7$, and $I = 21.4$. We obtained a second I-band image on March 8, UT 21 h 12 m with the same instrument on the WHT (exposure time 900 s), and a second V-band image on March 8, UT 20 h 42 min with the Isaac Newton Telescope (INT) on La Palma (exposure 2,500 s). Photometric calibration was obtained from images of standard star number 336 and Landolt²² field 104. The images were reduced using standard bias subtraction and flatfielding.

A comparison of the two image pairs immediately revealed one object with a large brightness variation⁶: it is clearly detected in both the V- and I-band images taken on 28 February, but not in the second pair of images taken on 8 March (Fig. 2). From a comparison with positions of nearby stars that were obtained using the Digitized Sky Survey we find for its location RA 05 h 01 min 46.66 s, dec. $+11^\circ 46' 53.9''$ (equinox J2000); this position has an estimated (internal) accuracy of 0.2". The object is located in the error box defined by the WFC position, the Ulysses/BeppoSAX/Wind annuli, and the transient X-ray source position (Fig. 1).

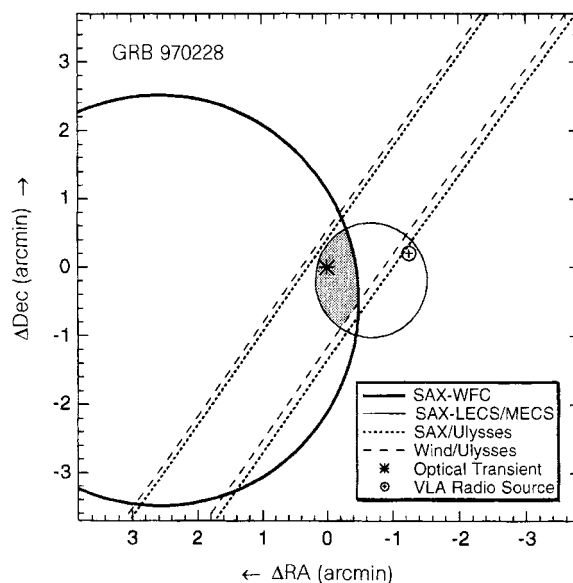


Figure 1 The position of the optical transient, indicated with an asterisk, is shown with respect to the 3' (radius) WFC location error circle, the 50" (radius) error circles of the BeppoSAX X-ray transient, and the two annuli obtained from the differences between the times the GRB was detected with Ulysses, and with BeppoSAX and Wind, respectively. The area in common between these error regions is hatched. The coordinates are given in units of arcmin with respect to the position of the optical transient (RA 05 h 01 min 46.66 s, dec. $+11^\circ 46' 53.9''$, J2000). The position of an unrelated radio source⁴⁹ in the error circle of the X-ray transient is indicated with the square symbol.

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Using aperture photometry software we determined the magnitudes of the variable as follows⁶: $V = 21.3 \pm 0.1$, $I = 20.6 \pm 0.1$ on 28 February and $V > 23.6$, $I > 22.2$ on 8 March. The shape of the source in both the 28 February V- and I-band images is consistent with that of the point-spread function, as determined for 15 stars in the same images.

Close to the optical transient is a star, located $2.85''$ away at RA 5 h 01 min 46.47 s, dec. $+11^\circ 46' 54.0''$, with $V = 23.1$, $I = 20.5$. A spectrum of this star, taken on March 1, UT 0 h with the ESO 3.6-m telescope using the EFOSC1 spectrograph and the R1000 grating (resolution of $14 \text{ \AA}$ per pixel), covering the $5,600\text{--}11,000 \text{ \AA}$ region, reveals the presence of TiO bands, which indicate it is an M-type star. With foreground absorption $A_v = 0.4 \pm 0.3 \text{ mag}$ (ref. 23), (substantially smaller than the value inferred from the low-energy cut off in the LECS spectrum), its colour index, $V - I = 2.6$, corresponds to an M2 star²⁴. It is most likely to be an M dwarf at a distance of $\sim 3 \text{ kpc}$ (an early M-type giant would be located at a distance of $\sim 0.4 \text{ Mpc}$, which we consider much less likely).

Further images were obtained with the Nordic Optical Telescope (NOT, La Palma) on 4 March, with the INT on 9 March, and with the ESO New Technology Telescope (NTT) on 13 March (see Table 1 for a summary). The transient was not detected in these images, which puts a lower limit on its average decay rate (in 4 days) of 0.7 mag per day . The NTT image shows that at the location of the variable object there is an extended object, probably a galaxy^{7,8} (Fig. 3); this object is also seen in the INT B- and R-band images. From differential astrometry relative to the nearby M star for both the V- and I-band images, we find that the centres of the optical transient and the galaxy have a relative distance ($0.22 \pm 0.12''$) (1σ ; quadratic addition of the errors in the two relative positions). The relative position of the optical transient did not change by more than $0.2''$ between the 28 February V- and I-band images. From the NTT image and the 9 March INT image we measured⁷ the galaxy's magnitude to be $R = 23.8 \pm 0.2$ and 24.0 ± 0.2 , respectively, consistent with the value $R = 24.0$ reported by Metzger *et al.*⁸, and $B = 25.4 \pm 0.4$ (Table 1).

Known types of optical transient events (novae, supernovae, dwarf novae, flare stars) are unlikely to account for the optical transient for a variety of reasons, such as the amplitude and short timescale of its variability, its colour index, or its inferred distance.

The GRB source is located relatively close to the ecliptic, at latitude -11° , and this raises the possibility that the optical transient is an asteroid. However, on 1 March asteroids in the direction of the GRB have proper motions of at least 0.1° per day (T. Gehrels, personal communication), which would have led to easily detectable motion ($>2.5''$) during the 600-s total exposure time of our two separate images. On the basis of its proper motion during

our two exposures we cannot rule out that the optical transient is a Kuiper belt object. However, its non-detection on other images taken in the week following the GRB, the low surface density (one per several hundred square degrees at 21st magnitude; T. Gehrels, personal communication) of Kuiper belt objects, and their very red colours²⁵, make such objects a highly unlikely explanation of the optical transient.

The variability of the optical sky above 21st magnitude, on timescales of a few days, has not yet been extensively explored^{26,27}, and it is therefore not possible to make a firm estimate of the probability that the optical transient we detected is a faint, strongly variable AGN unrelated to the GRB, or has another (unknown) origin unrelated to the GRB. Some information is available from the faint-galaxy monitoring program of Kochanski *et al.*²⁸ which covered 2,830 galaxies (down to $B = 24.8$, $R = 23.3$) in a $16' \times 16'$ field during 10 years. They found that near $B = 24$ only $\sim 0.5\%$ of these varied by more than 0.03 mag on a timescale of months to years; none varied by more than a magnitude. Variability of blazars on timescales of minutes to hours does not exceed 0.3 mag ; day-to-day variations of a factor of two or more have been observed³⁰.

Although we cannot firmly exclude that the optical transient in the error box of GRB970228 is caused by some unknown event unrelated to the GRB, the temporal coincidence between the optical and X-ray transients, and their spatial coincidence with the GRB lead us to believe that both the optical transient and the decaying BeppoSAX X-ray source are associated with GRB970228.

Radio observations (at 6 cm wavelength) of the GRB error box⁶ made with the Westerbork Radio Synthesis telescope on February 28, UT 23 h 17 min (for 1.2 h; 20.4 h after the GRB, simultaneous with the observations at the WHT), and on March 1, UT 18 h and March 2, UT 18 h (each lasting 12 hours) show that at the position of the optical transient there is then no radio point source with a flux exceeding 1.0 , 0.33 and 0.33 mJy , respectively (2σ upper limit).

Some rough spectral information on the optical/X-ray transient can be obtained if we assume that between the two BeppoSAX X-ray observations, made 4 days apart, the X-ray flux of the transient decreased with time since the GRB as a power law. Approximating the spectrum by $F_\nu \propto \nu^\alpha$ we estimate from $F_x \approx 0.04 \text{ } \mu\text{Jy}$ (interpolated) and $F_v \approx 10 \text{ } \mu\text{Jy}$, that $\alpha = -0.7 \pm 0.1$. Extrapolation of this spectrum to the radio region would lead to an expected radio flux density of $10\text{--}100 \text{ mJy}$, far exceeding the observed upper limit. This indicates that the X-ray, optical and radio flux densities of the transient cannot be represented by a single power law.

The close positional coincidence between the optical transient and the galaxy suggests that the transient may be located in that galaxy. In an effort to quantify this we adopt a bayesian approach. We consider three disjoint and exhaustive hypotheses for the source

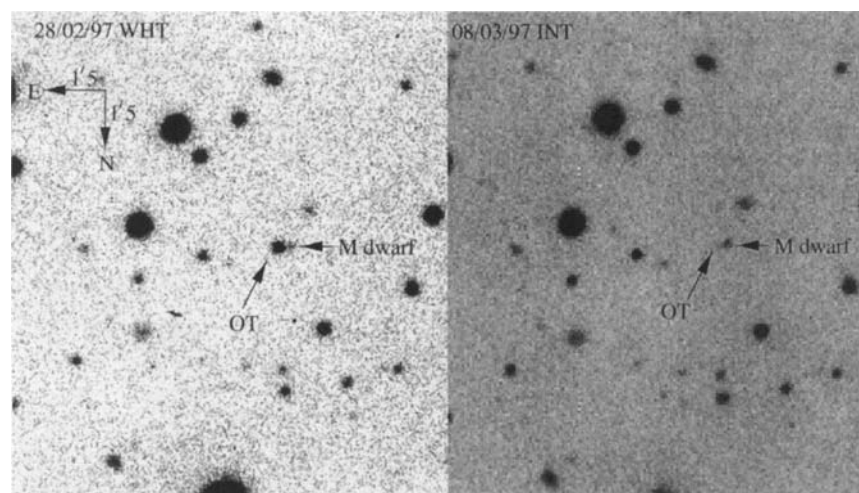


Figure 2 V-band images of a $1.5' \times 1.5'$ region of the sky containing the position of the optical transient. The left image was obtained with the WHT on 1997 28 February, UT 23 h 48 min, the right image with the INT on 8 March, UT 20 h 42 min. The optical transient is indicated by 'OT'. The M dwarf, separated from the optical transient by $2.9''$, is also indicated.

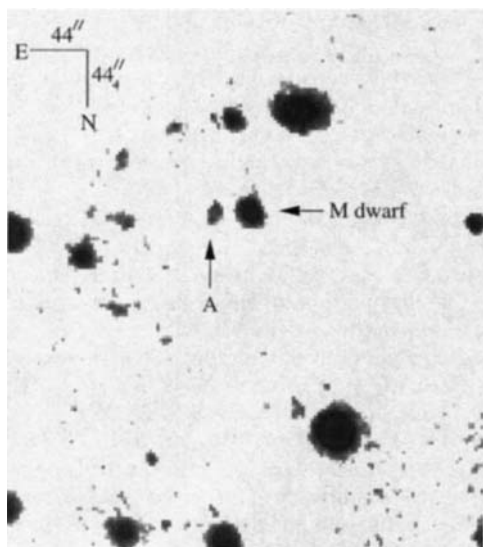


Figure 3 R-band image of a 44'' × 44'' region of the sky containing the position of the optical transient, obtained with the NTT on 1997 13 March ut 0h. The faint galaxy coincident with the optical transient (A) and the M-dwarf are indicated.

of the optical transient. The hypotheses are: H_c , the optical transient is in the centre of a galaxy, H_g , the optical transient is in a galaxy but not at its centre; and H_n , the optical transient is not in a galaxy.

We assume that there is a single optical transient detected in the field of view of angular area A and that n non-overlapping galaxies are detected in the field. The transient is at distance $r \pm \sigma_r$ from the nearest galaxy, where the error includes the uncertainty in the positions of the centroids of both the galaxy and the transient. The probability density at r under H_c is $P(r|H_c) = (2\pi\sigma_r^2)^{-1} \exp[-(r^2/2\sigma_r^2)]$. The probability density under H_g depends on the size, shape and inclination of the galaxy and the specifics of the model for the distribution of sources in the galaxy. For simplicity, we assume the probability density to be gaussian with width σ_g . Then $P(r|H_g) = (2\pi\sigma_g^2)^{-1} \exp[-(r^2/2\sigma_g^2)]$. The probability density under H_n is uniform over the field of view, so $P(r|H_n) = A^{-1}$. The posterior probability of each hypothesis H is $P(H|r) = kP(H)P(r|H)$, where $P(H)$ is the prior probability and the normalization constant k is obtained by the requirement that $P(H_c|r) + P(H_g|r) + P(H_n|r) = 1$.

For the NTT observation we find seven galaxies in $A = (44'')^2$ field, that is, $n = 13$ per arcmin², $r = 0.22''$, $\sigma_r = 0.12''$. A reasonable estimate for the galaxy width is $\sigma_g = 1''$. With these values the probability densities at r are $P(r|H_c) = 0.294$, $P(r|H_g) = 0.022$ and $P(r|H_n) = 0.0005$, all in units of arcsec⁻². Assuming equal priors $P(H_c) = P(H_g) = P(H_n) = 1/3$, the posterior probabilities are $P(H_c|r) = 0.928$, $P(H_g|r) = 0.070$ and $P(H_n|r) = 0.0016$. The posterior probability for H_g depends sensitively on the assumed σ_g . However, the posterior probability, $P(H_n|r)$, that the transient is not associated with a galaxy is in the range 0.09–0.18%, for any assumptions about the size of faint galaxies. Within the range of assumed values for σ_r , between 0.08'' and 0.2'' the values of $P(H_n|r)$ increase by less than a factor of 3.

The above analysis suggests that the optical transient is related to the faint galaxy, which provides support for the cosmological distance scale for GRBs.

A rough estimate of the expected redshift, z , of the galaxy may be made by assuming that its absolute magnitude is in the range -21 to -16 , which covers the bulk of normal galaxies²⁹. For an assumed Hubble constant of 60 km s⁻¹ Mpc⁻¹, this corresponds to z in the range 0.2–2.

The close proximity of the optical transient to the centre of the faint galaxy, and the presence of relatively bright quasars in the

Table 1 Summary of optical observations

Date (1977)	Time (ut)	Telescope	Band	Integration time (s)	Magnitude	Remarks
28 Feb.	23 h 48 min	WHT	V	300	$V = 21.3$	Transient
28 Feb.	23 h 53 min	WHT	I	300	$I = 20.6$	Transient
04 Mar.	20 h 42 min	NOT	V	900	$V > 24.2$	–
08 Mar.	20 h 42 min	INT	V	2,500	$V > 23.6$	–
08 Mar.	21 h 12 min	WHT	I	300	$V > 22.2$	–
09 Mar.	21 h 30 min	INT	R	1,200	$R = 24.0$	Extended
09 Mar.	20 h 30 min	INT	B	2,500	$B = 25.4$	Extended
13 Mar.	0 h 0 min	NTT	R	3,600	$R = 23.8$	Extended

8 arcmin² error box of GRB781119 ($V = 20$)^{30–32}, and in the 3' (radius) error box³³ of GRB960720 ($R = 18.8$)^{34–36} raise the possibility that GRBs occur preferentially, or exclusively, in or near galactic nuclei.

Searches for an optical counterpart to a GRB have been continually attempted for the past 20 years. Recent reviews and descriptions of serendipitous, rapid follow-up, and delayed searches for optical counterparts of GRBs^{36–44} show that these previous searches were generally made a week or longer after the GRB, or they were not as deep ($V < 20$) as the images presented here. The most sensitive rapid follow-up observations so far had delay times (δt) and limiting magnitudes (m) as follows: $\delta t = 1.85$ d, $m < 23$ (ref. 45), and $\delta t = 4.0$ d, $m_B < 22$ (ref. 38).

It was not until the launch of BeppoSAX in 1996 that accurate (several arcmin) locations for GRBs became available within hours of detection, hence facilitating rapid follow-up observations at large ground-based optical telescopes for those bursts which happened to be in the field of the WFC. The continued operation of BeppoSAX and the approval of the High Energy Transient Explorer-2 (HETE-2) mission bode well for great progress in the rapid follow-up observations of GRBs. Also, near-real-time, fully automated optical systems linked to the BATSE-BACODINE³⁷ system are becoming operational, and their sensitivity is continually improving^{46,47}.

We expect that X-ray and optical transients associated with GRBs will again be seen (though perhaps not in all cases⁴⁸) in the near future. This could be a turning point in GRB astronomy. Detailed studies (light curves and spectra) of such transients can be expected within a year, and we are optimistic that the distance scale as well as the mechanism behind the enigmatic GRBs are now within reach. *Note added in proof:* After this paper was submitted, an HST observation was made of the optical transient (K. Sahu *et al.*, *IAU Circ. No. 6606*). This observation confirms that the transient is associated with an extended emission region, but seems to exclude that the transient is located at the centre of that region. □

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Momentum creation by vortices in superfluid ³He as a model of primordial baryogenesis

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The Universe contains much more matter than antimatter, which is probably the result of processes in the early Universe in which baryon number was not conserved. These processes may have occurred during the electroweak phase transition, when elementary particles first acquired mass^{1–4}. It is impossible to study

directly processes relevant to the early Universe, because of the extreme energies involved. One is therefore forced to investigate laboratory systems with analogous phase transitions. Much of the behaviour of superfluid ³He is analogous to that predicted within the standard model of the electroweak interaction⁵. Superfluids and liquid crystals have already been used to investigate cosmic-string production^{6–11}; here we describe experiments on ³He that demonstrate the creation of excitation momentum (which we call momentogenesis) by quantized vortices in the superfluid. The underlying physics of this process is similar to that associated with the creation of baryons within cosmic strings, and our results provide quantitative support for this type of baryogenesis.

To explain the creation of matter in the early Universe we begin by recalling the relationship $E^2 = m_0^2 c^4 + p^2 c^2$ between energy E and momentum p for relativistic particles with rest mass m_0 (c is the velocity of light). Dirac realized that the square root of this equation, $E = \pm (m_0^2 c^4 + p^2 c^2)^{1/2}$ produced particles with both positive and negative energy. This led to his famous picture of the vacuum state (the state with no real particles) as one in which all the negative energy states are full and all the positive energy states are empty. A real particle is then created as shown in Fig. 1a by excitation of a particle from the 'Dirac sea' of negative-energy states to a positive-energy state. But such processes create matter and antimatter in equal amounts, as the appearance of a hole in the negative energy states is interpreted as the simultaneous creation of an antiparticle.

The net creation of matter in the form of baryons such as protons and neutrons requires processes in which antiparticles are not simultaneously created and thus baryon number is not conserved. In the 'standard model' the baryon number is classically conserved but can be violated by quantum-mechanical effects known generically as 'chiral anomalies'. The process leading to matter creation is called 'spectral flow' and can be pictured as a process in which particles flow from negative energies to positive energies under the influence of an external force. In this way real observable positive-energy particles can appear without simultaneous creation of antiparticles. Figure 1b illustrates a simple example of spectral flow occurring for massless particles with electric charge q (in units of the charge of the electron, e) moving in a magnetic field. The application of an electric field E leads to the production of particles from the vacuum at the rate

$$\dot{n} = \partial_\mu j^\mu = \frac{q^2}{4\pi^2} \mathbf{E} \cdot \mathbf{B} \quad (1)$$

per unit volume, where j is the particle current fourvector; factors of ($e\hbar$) have been absorbed in the definition of the electric and magnetic fields. This is an anomaly equation for the production of particles from the vacuum of the type found by Adler¹² and by Bell and Jackiw¹³ in the context of neutral pion decay. We see that for particle creation it is necessary to have an asymmetric branch of the dispersion relation $E(p)$ which crosses the axis from negative to positive energy. We call such a branch a zero mode branch; a spectrum of this type was first found for vortex core excitations in a superconductor¹⁴.

Similar zero mode branches exist on a cosmic electroweak string (also known as a Z-string), which is a structure of the Higgs field that may have been produced during the electroweak phase transition. The Higgs field gives the particles mass outside the string core. This field vanishes on the string axis and the fermions (quarks and leptons) occurring in the core of the string behave like massless one-dimensional particles. Spectral flow on a Z-string leading to production of baryons from the vacuum with conservation of electric charge is illustrated in Fig. 1c. Motion of the string across a background electromagnetic field¹⁵ or the de-linking of two linked loops^{16,17} provides a mechanism for cosmological baryogenesis¹⁸ and could lead to the presence of antimatter in cosmic rays¹⁹.

We should point out that baryon-number violation is only one

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ingredient in a cosmological baryogenesis model. The other ingredients are that the system has to be out of thermal equilibrium, and charge and charge-parity conjugation symmetries should be violated; these conditions are known as the 'Sakharov conditions' for cosmological baryogenesis²⁰. In condensed matter the analogous symmetry breaking is provided by rotation (for ^3He) or a magnetic field (for superconductors), and disequilibrium is provided by the motion of vortex lines. We shall see that excitation momentum is the analogue of baryon number.

The superfluidity of ^3He is due to the formation of bound pairs of ^3He atoms known as Cooper pairs. There are two main superfluid phases, A and B, which have different Cooper-pair wavefunctions. At the lowest temperature, the superfluid vacuum state is obtained in which all the atoms are Cooper-paired. By analogy with the Dirac picture of the vacuum (Fig. 1a) the Cooper-paired atoms are seen as filling all the negative energy states and are separated from the world of normal particles by the Cooper-pair binding energy. The Cooper-pair wavefunction (usually called the order parameter) which is responsible for this energy gap is thus analogous to the Higgs field in electroweak theory, as it is the finite particle mass produced by the Higgs field that results in the energy gap in electroweak theory between the negative- and positive-energy states in Fig. 1a. At higher temperatures in ^3He some atoms are excited from the ground state by the breaking of Cooper pairs and these normal (non-superfluid) excitations interact with the order parameter in a way that is similar to the interaction of the standard model particles with the electroweak gauge and Higgs fields. Thus the behaviour of leptons and quarks on fixed gauge and Higgs field backgrounds can be modelled by the behaviour of ^3He excitations on fixed order parameter backgrounds such as quantized vortices. In ^3He a zero-mode branch exists for particles in the core of quantized vortices (Fig. 2a). A physically important charge in ^3He -A, ^3He -B and superconductors, which (like baryonic charge in the standard model) is not conserved due to the anomaly, is excitation momentum. Spectral flow along the zero-mode branch leads to an additional 'lift' force on a moving vortex (Fig. 2b, the analogue of Fig. 1c).

This is most easily seen for the doubly quantized continuous vortex (winding number $N = 2$) in the A-phase of superfluid ^3He , which is the closest analogue of a Z-string. The Cooper pairs in ^3He -

A have angular momentum $\hbar$ and locally all the pairs have this angular momentum aligned along a direction $\hat{\mathbf{l}}$. The $N = 2$ vortex is characterized by a continuous distribution of the order parameter vector $\hat{\mathbf{l}}$ as shown in Fig. 2c. The time- and space-dependent $\hat{\mathbf{l}}$ vector associated with the motion of the vortex produces a force on the excitations equivalent to that of an 'electric field' $\mathbf{E} = k_F \partial_t \hat{\mathbf{l}}$ and a 'magnetic field' $\mathbf{B} = k_F \nabla \times \hat{\mathbf{l}}$ acting on particles of unit charge, where $k_F = p_F/\hbar$ and p_F is the Fermi momentum. Equation (1) can then be applied to calculate the rate at which left-handed quasi-particles and right-handed quasiholes are created by spectral flow. As both types of excitation have momentum $p_F \hat{\mathbf{l}}$, excitation momentum is created at a rate:

$$\partial_t \mathbf{P} = \frac{1}{2\pi^2} \int d^3 r p_F \hat{\mathbf{l}} (\mathbf{E} \cdot \mathbf{B}) \quad (2)$$

However, total linear momentum must be conserved. Therefore equation (2) means that, in the presence of a time-dependent texture, momentum is transferred from the superfluid ground state (analogue of vacuum) to the heat bath of excitations forming the normal component (analogue of matter).

Integration of the anomalous momentum transfer in equation (2) over the cross-section of the moving vortex gives the loss of linear momentum and thus the additional force per unit length acting on the vortex due to spectral flow:

$$\mathbf{F}_{sf} = \partial_t \mathbf{P} = \pi \hbar N C_0 \hat{\mathbf{z}} \times (\mathbf{v}_n - \mathbf{v}_L) \quad (3)$$

Here $\hat{\mathbf{z}}$ is the direction of the vortex, $C_0 = k_F^2/3\pi^2$, $\mathbf{v}_L$ is the velocity of the vortex line, $\mathbf{v}_n$ is the heat bath velocity, and N is the winding number of the vortex.

The insets to Fig. 3a show how the force on a vortex is measured experimentally. A uniform array of vortices is produced by rotating the whole cryostat, and oscillatory superflow perpendicular to the rotation axis is produced by a vibrating diaphragm, while the normal fluid (thermal excitations) is clamped by viscosity. The velocity $\mathbf{v}_L$ of the vortex array is determined by the overall balance of forces acting on the vortices, conventionally written as^{21,22}

$$n_s \pi \hbar N [\hat{\mathbf{z}} \times (\mathbf{v}_L - \mathbf{v}_s) + d_\perp \hat{\mathbf{z}} \times (\mathbf{v}_n - \mathbf{v}_L) + d_\parallel (\mathbf{v}_n - \mathbf{v}_L)] = 0 \quad (4)$$

where $n_s(T)$ and $\mathbf{v}_s$ are the density and velocity of the superfluid

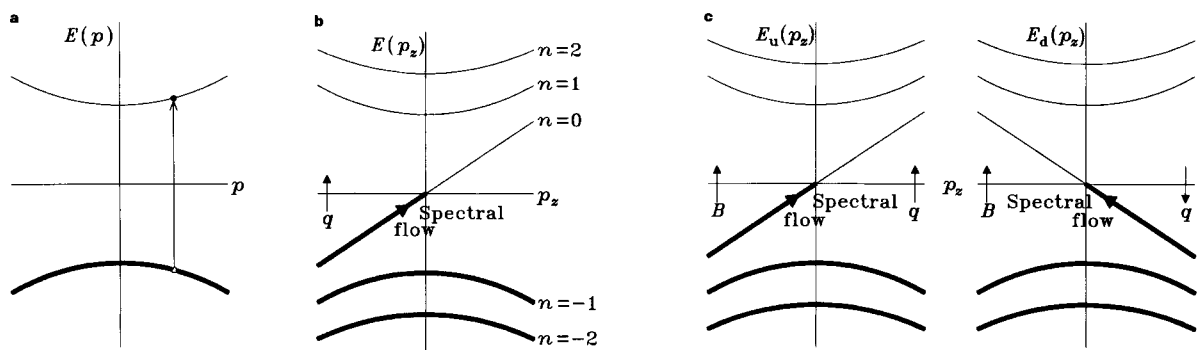


Figure 1 **a**, Particles and antiparticles of energy E and momentum p in the Dirac picture; the thick line shows occupied negative-energy states. Promotion of a particle from negative energy to positive energy creates a particle-antiparticle pair from the vacuum. **b**, Spectrum of massless right-handed particles in a magnetic field $\mathbf{B}$ along z ; the thick lines show the occupied negative-energy states. The right-handed chirality of the particle means that their spin is aligned with their linear momentum. Motion of the particles in the plane perpendicular to $\mathbf{B}$ is quantized into the Landau levels shown. The free motion is thus effectively reduced to one-dimensional motion along $\mathbf{B}$ with momentum p_z . Because of the chirality of the particles the lowest ($n = 0$) Landau level, for which $E = cp_z$, is asymmetric: it crosses zero only in one direction. If we now apply an electric field $\mathbf{E}$ along z , particles of charge q are pushed from negative- to positive-energy levels according to the equation of motion $\dot{p}_z = qE$, and the whole Dirac sea (see

text) moves up, creating particles and electric charge from the vacuum. This motion of the particles along the 'anomalous' branch of the spectrum is called spectral flow. The rate of particle production is proportional to the density of states at the Landau level, which is $\propto q|\mathbf{B}|$, so that the rate of production of particles from the vacuum $\propto q^2 \mathbf{E} \cdot \mathbf{B}$. **c**, Anomalous branches of the spectrum of u-quarks ($q = +2/3$; left panel) and d-quarks ($q = -1/3$; right panel) in the core of a Z-string. There are anomalous branches for electrons ($q = -1$) and neutrinos ($q = 0$) also. The neutrino and u-quark propagate in one direction along the string while the electron and d-quark propagate in the opposite direction. For every electron produced, two u-quarks and one d-quark are created so that there is no net production of electric charge but the baryon number B increases by one (2 u-quarks + 1 d-quark = 1 proton = 1 baryon).

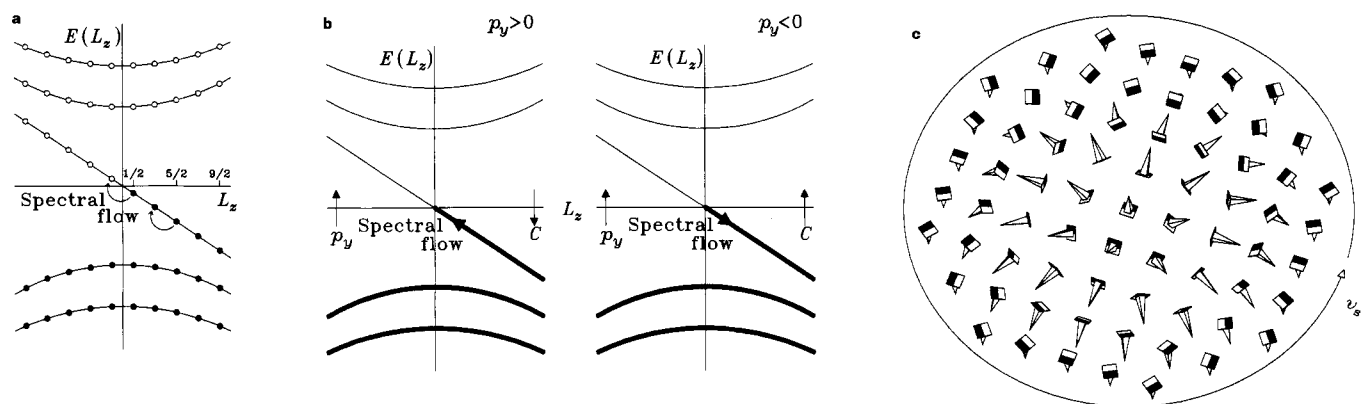


Figure 2 a, Spectrum of particles in the core of vortices in ^3He and superconductors; filled circles show occupied states. The only difference from the Z-string is that the anomalous branch, $E_3(p_z, L_z) = -L_z \hbar \omega_c(p_z)$, of the spectrum crosses zero as a function of the discrete angular momentum $\hbar L_z$, where L_z is half an odd integer. Typically the interlevel distance $\hbar \omega_c$ is very small compared to the characteristic energy scales in superconductors and Fermi superfluids, and can be comparable to the level width $\hbar/\tau$ resulting from the scattering of core excitations by free excitations in the heat bath outside the core. If $\omega_c \tau < 1$, the levels overlap and spectral flow is allowed. This type of spectral flow is analogous to 'hopping conduction' in a solid; it is assisted rather than impeded by collisions. **b**, Such spectral flow is induced by the motion of the vortex with respect to the heat bath. If the vortex moves with velocity v_x along x , the rate of change of excitation angular momentum in the moving core is $L_z = x p_y = v_x p_z$. The levels

cross zero at a rate $-L_z/\hbar$ leading to spectral flow in opposite senses for $p_y > 0$ and $p_y < 0$ as indicated. As the left-handed quasiparticles created for $p_y > 0$ are equal in number to the right-handed quasiholes created for $p_y < 0$ there is no net production of chiral charge C , but excitation momentum is created at a rate $p_y = -v_x p_z/\hbar$, independent of the sign of p_y (shown for negative v_x). **c**, An example of an $N = 2$ continuous vortex in $^3\text{He-A}$. The cones indicate the local direction of the order parameter vector $\hat{\mathbf{i}}$, which is parallel to the angular momentum of the Cooper pairs, and is analogous to the direction of the isotopic spin in electroweak theory. Rotation of the cones about $\hat{\mathbf{i}}$ indicates a change in phase of the order parameter; note the 4π phase change around the perimeter of the diagram, corresponding to two quanta of anticlockwise circulation. Four topologically different types of $^3\text{He-A}$ vortices have been observed²².

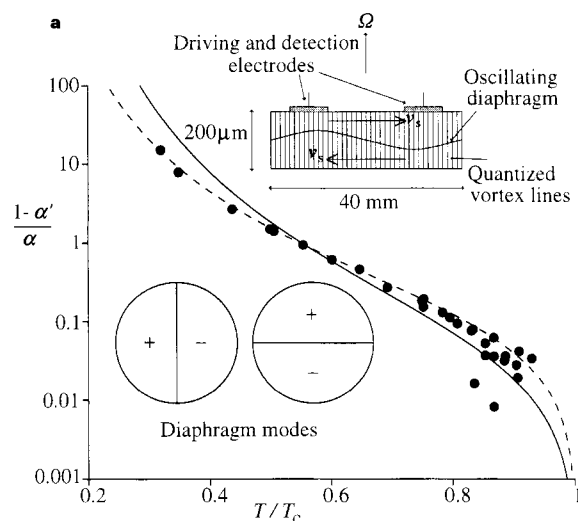
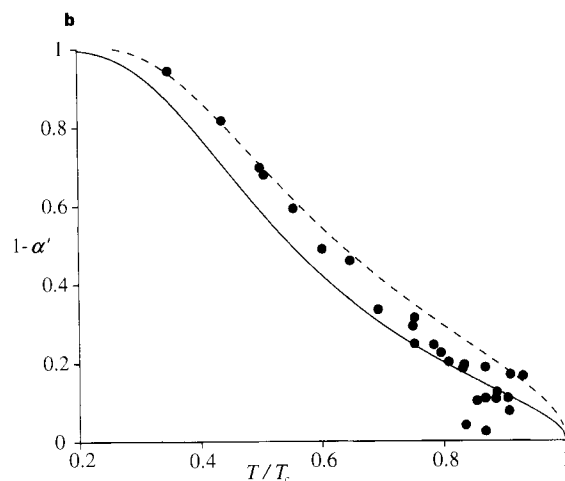


Figure 3 a, The experimental cell is shown in the top inset. The aluminized Kapton film diaphragm separates two disk-shaped regions of superfluid ^3He , each 100 μm thick. The roof of the cell has six electrodes set into it by means of which the oscillations of the diaphragm may be driven and detected electrostatically. In the oscillating modes of interest the motion of the diaphragm displaces the superfluid as indicated, while the normal component of the fluid (the heat bath) is clamped by its high viscosity. Rotation at angular velocity Ω about a vertical axis produces vortices normal to the diaphragm. These vortices produce additional dissipation proportional to $\alpha \Omega$ and coupling between two orthogonal modes of the diaphragm, with displacement patterns shown in the bottom inset, proportional to $(1 - \alpha')\Omega$. The measured spectral flow parameters are related to the parameters in ref. 22 by $\alpha = B n_c/2n$ and $\alpha' = B' n_c/2n$.



Main figure, the measured $(1 - \alpha')/\alpha$ for $^3\text{He-B}$ at 20 bar is compared with the theoretical value $(1 - \alpha')/\alpha \approx \omega_3 \tau$. The temperature dependence of $\omega_3 \tau$ is not very well known, as it is sensitive to the details of the quasiparticle scattering. It can be estimated by the formula²⁷ $\omega_3 \tau = (a \Delta(T)/k_B(T)^2 \exp(\Delta(T)/k_B T))$. The solid line is for $a = 0.067$. The fit is much improved if an effective energy gap 0.6 of the bulk value is assumed; the dashed line is for $\Delta(T) = 0.6 \Delta_{0,K}(T)$ and $a = 0.214$. But the justification for such an assumption is not clear. **b**, The experimental parameter $(1 - \alpha')/\alpha$ for $^3\text{He-B}$ at 20 bar is compared with the 'spectral flow' prediction of eq. (5): $(1 - \alpha')/\alpha \approx n_s(T)/(n \tanh(\Delta(T)/2k_B T))$ (solid line). Note that this fit is independent of the relaxation parameter $\omega_3 \tau$. The dashed line is for $\Delta(T) = 0.6 \Delta_{0,K}(T)$, and fits much better below $0.6 T_c$. This fit shows that spectral flow is less strongly suppressed at low temperatures than the full energy gap would suggest.

component. The first term is the Magnus force, which appears when the vortex moves with respect to the superfluid, and the terms with $(\mathbf{v}_n - \mathbf{v}_l)$ represent the nondissipative transverse and frictional longitudinal forces, proportional to dimensionless parameters $d_\perp$ and $d_\parallel$ respectively, which appear if the vortex moves with respect to the normal fluid. The diaphragm has to provide a force equal and opposite to the Magnus force to drive the superfluid. Measurement of the damping of the diaphragm resonance and of the coupling between the two orthogonal modes illustrated in Fig. 3a enables both $d_\perp$ and $d_\parallel$ to be deduced.

For the A-phase, the spectral flow force in equation (3) combines with the Iordanskii force²¹ to give $d_\perp \approx (C_0 - n_n)/n_s$, where $n_n(T) = n - n_s(T)$ is the density of the normal component, with n the total density. As $C_0 \approx n$, one has $d_\perp \approx 1$. Our ³He-A experiments made at 29.3 bar and $T > 0.82T_c$, where T_c is the superfluid transition temperature, are consistent with this within experimental uncertainty: we find that $|1 - d_\perp| < 0.005$ (ref. 23).

Any Fermi superfluid becomes similar to the A-phase in the vicinity of the vortex core. However, in ³He-B the spacing $\hbar\omega_0$ between bound states on the anomalous branch in Fig. 2a becomes larger than the broadening $\hbar\tau^{-1}$ due to the lifetime τ at low T (ref. 21). There should thus be a transition from full spectral flow as $T \rightarrow T_c$ to totally suppressed spectral flow as $T \rightarrow 0$, a further aspect of the theory which can be tested experimentally. The interpolation formula as a function of the relaxation parameter $\omega_0\tau$ and the spectral flow parameter $C_0 \approx n$ (refs 21, 24) can be written in the form

$$d_\parallel - i(1 - d_\perp) = \frac{1}{\alpha + i(1 - \alpha')} = \frac{n}{n_s} \frac{\omega_0\tau}{1 + i\omega_0\tau} \tanh \frac{\Delta(T)}{2k_B T} \quad (5)$$

where $i = (-1)^{1/2}$ and the α parameters are what are directly measured experimentally²². The experimental results are compared with equation (5) in Fig. 3. The agreement is excellent in view of the approximations in the theory.

Our results thus show that the chiral anomaly is relevant for the interaction of condensed matter vortices (analogous to strings) with fermionic excitations (analogous to quarks and leptons); this gives a firmer footing to chiral anomaly calculations for baryogenesis on non-trivial backgrounds of the Higgs field such as cosmic strings. Superfluid ³He is the most complex field-theoretical system available in the laboratory, and is therefore the closest experimental analogue of the field-theoretical foundations of the physics of the early Universe. It is characteristic of nonlinear theories that experiment often reveals qualitatively new phenomena that would be hard to find by consideration of the equations alone. We may therefore hope that imaginative experiments on superfluid ³He will generate new ideas in cosmology. □

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Morphogenesis of shapes and surface patterns in mesoporous silica

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The synthesis of inorganic materials with complex form, using surfactant assemblies as supramolecular templates^{1–13}, has ramifications in areas as diverse as large-molecule catalysis, the formation of semiconductor nanostructures, biomolecule separations, the development of medical implants and the morphogenesis of skeletal forms¹⁴. Here we describe a procedure for the synthesis of hexagonal mesoporous silica that produces a remarkable array of shapes, surface patterns and channel plans. Our reaction conditions favour curved morphologies including toroidal, disk-like, spiral and spheroidal shapes. We use scanning electron microscopy to catalogue the basic topologies and surface patterns, and transmission electron microscopy to establish the relationship between morphology and the underlying mesostructure. Polarized optical microscopy enables us to identify a connection between optical anisotropy in these structures and the periodic porous mesostructure. We propose that the morphogenesis of these shapes and surface patterns can be rationalized in terms of the growth of a silicate liquid-crystal embryo^{15,16} with a hexagonal cross-section that, under different initial reaction conditions, is subject to increasing degrees of curvature.

We synthesized these mesoporous silica bodies under quiescent aqueous acidic conditions using cetyltrimethylammonium chloride (CTACl) as surfactant template and tetraethylorthosilicate (TEOS) as silica precursor^{10,13,17}. The materials obtained are ordinary in that they have the well documented hexagonal symmetry channel structure of mesoporous MCM-41², as signalled by the commonality of their diagnostic powder X-ray diffraction (PXRD) pattern^{13,17}. On the other hand, scanning electron microscopy (SEM) images reveal that the morphologies are extraordinary in terms of their diverse and remarkable shapes and impressive range of curvatures (Fig. 1). High-resolution SEM images of the exterior surfaces of the curved mesoporous silica morphologies show that they tend to fall into two classes: some are smooth at the 200–500-Å length scale but with signs of stepped and spiral growth, whereas others display faceted corrugated patterns that emanate radially or

spirally from the topologically unique rotation axis. It is important to note that the rope shapes typically have a curved and twisted body based on an elongated hexagonal cylinder, display both arc and crankshaft body forms, show well-defined faceting associated with body bending, and exhibit hexagonal basal faces. This faceting implies that the hexagonal 'ropes' may extend in size by the accretion of surfactant-silicate micelles on the basal planes. Furthermore, we found that certain ranges of concentration favour low-curvature ropes and related shapes, while others promote larger-curvature gyroids, spheroids and related forms (Fig. 2).

Transmission electron microscopy (TEM) images (microtomed 100–300-Å thin sections, recorded on a Philips 430 microscope operating at an accelerating voltage of 100 kV) of the basic

morphologies clearly depict the presence of hexagonally close-packed channels with a centre-to-centre spacing of ~ 50 Å. These images establish the spatial relationship between the channel plan and the body shape of the mesoporous silica morphologies. The channels that run down the body length of the ropes and wind around the loops, bracelets and toroids, also run circumferentially about the discoids, gyroids and related forms.

With this information we deduce that by increasing the degree of curvature of the faceted rope morphologies, it should be possible to generate discoids and gyroids with periodic faceting on their outer surfaces. Torsional stresses that might occur during this growth process could result in the observed pinwheel and shell-like shapes, exhibiting similar surface faceting. The surface corrugations of a

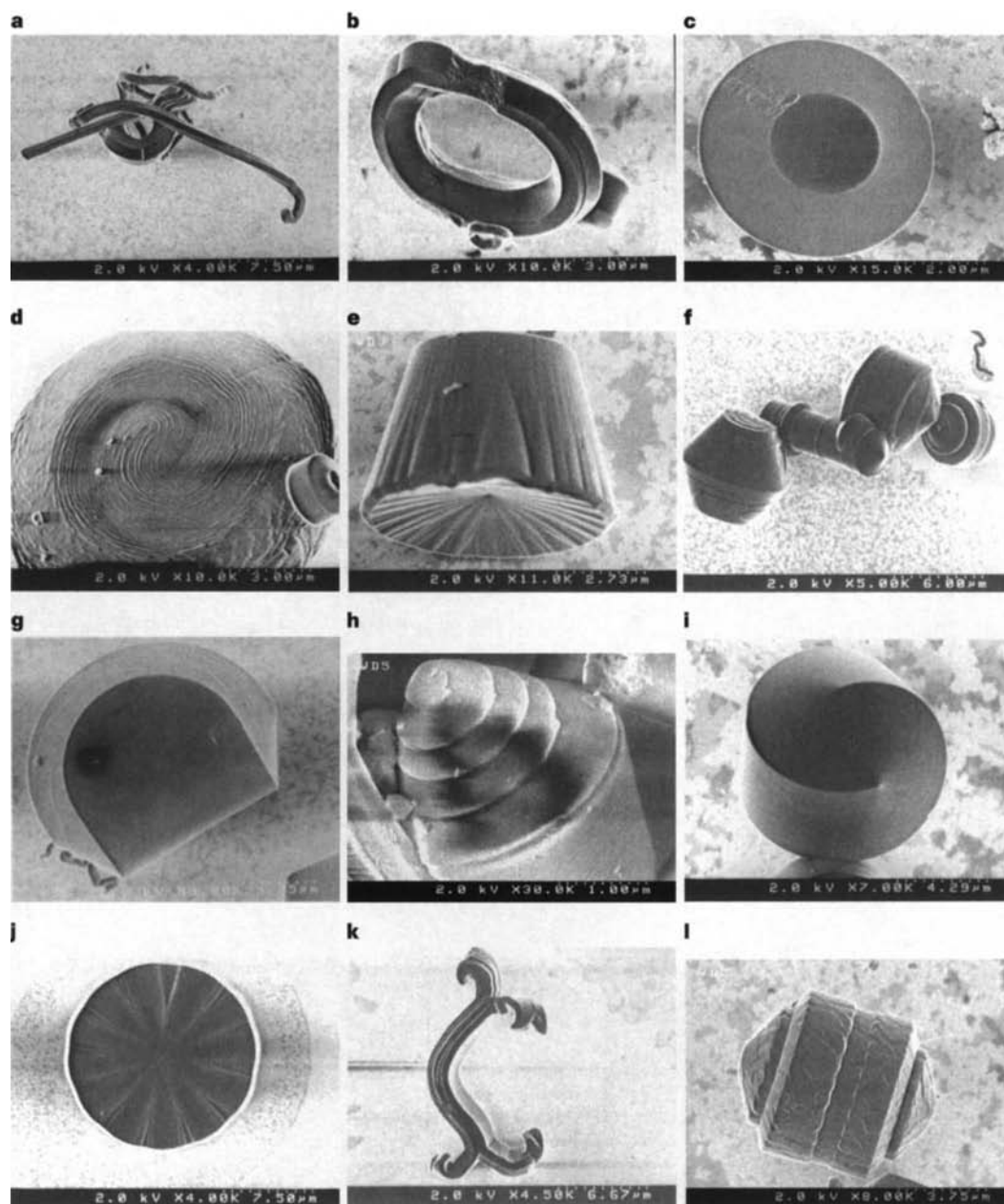


Figure 1 SEM images (obtained on a Hitachi S-4500 field emission microscope using a low acceleration voltage of 2 kV to minimize the charging of the mesoporous silica surfaces) of representative mesoporous silica shapes and

surface patterns: **a**, rope; **b**, toroid; **c**, discoid; **d**, pinwheel; **e**, wheel; **f**, gyroid; **g**, bagel; **h**, shell; **i**, knot; **j**, clock; **k**, eccentric 1; and **l**, eccentric 2. Note that dark areas appear on some of the images, owing to surface charging effects.

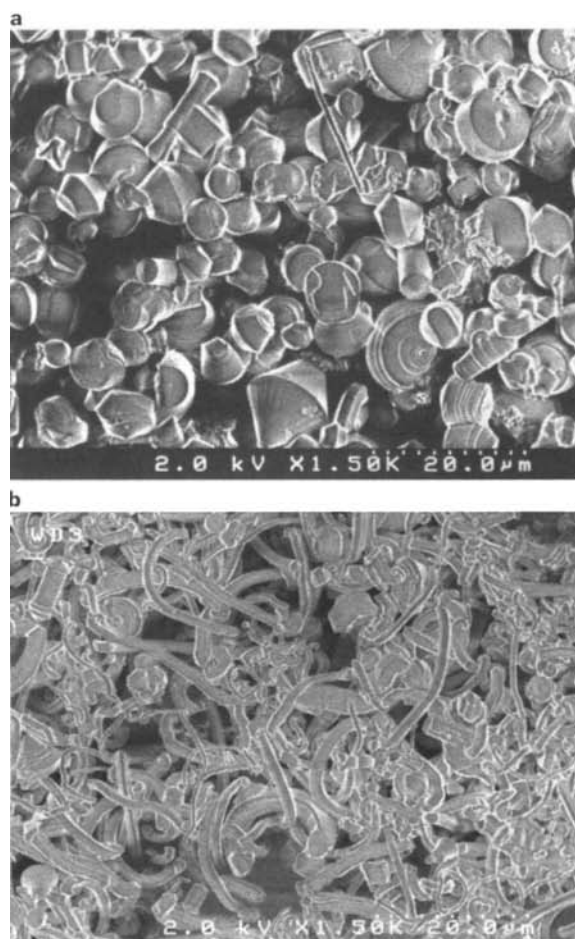


Figure 2 SEM images of the product obtained by using 100 H₂O, n HCl, 0.1 CTACl, 0.1 TEOS. **a**, $n = 3.5$; **b**, $n = 7$.

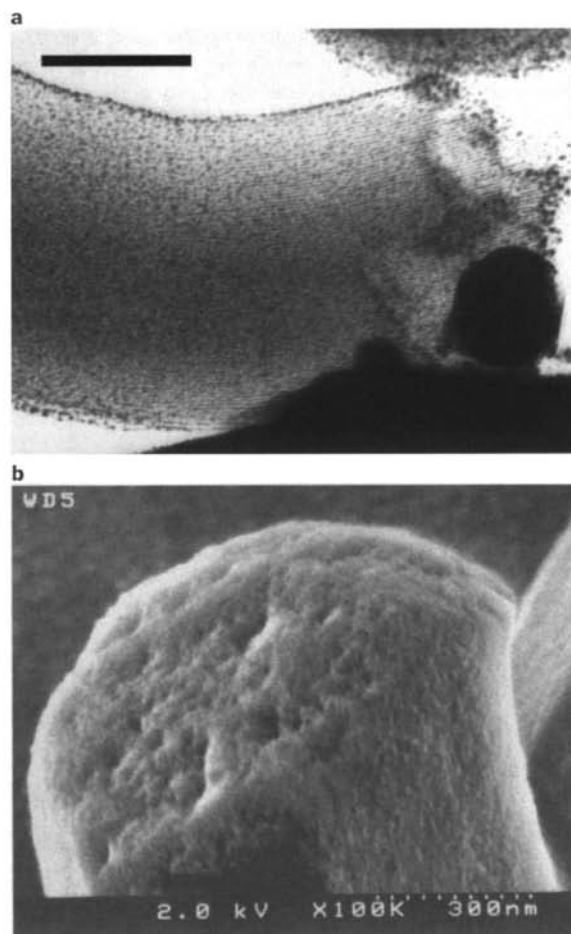


Figure 4 a, TEM image, recorded in transmission mode for a rope morphology, revealing the 25-Å spacing of curving parallel aligned channels (viewed along the (210) direction), as well as the structure at the growth fronts in the head and body regions. Scale bar, 125 nm. **b**, Representative SEM image of the head region of a rope.

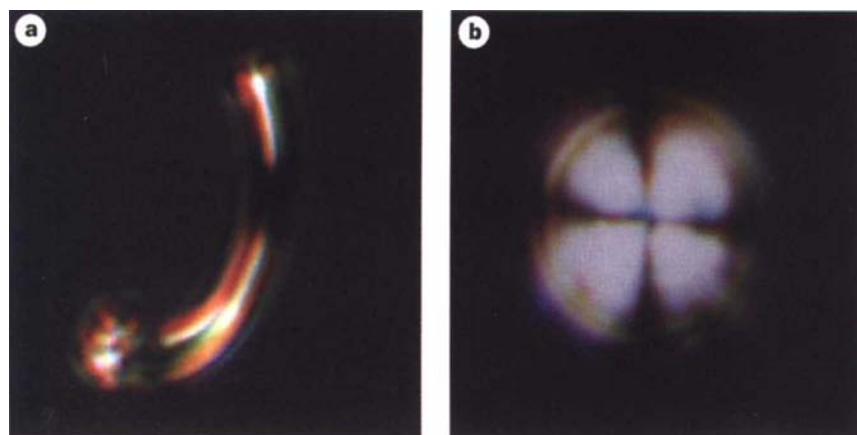


Figure 3 POM images (recorded on an Olympus BH-2 microscope, in convergent white light and between parallel and crossed polars) of representative rope (**a**) and discoid (**b**) mesoporous silica morphologies.

particular form will presumably depend on the dimensions, rate of growth and curvature of the rope-shaped precursor (Fig. 1). There exists a small group of mesoporous silica morphologies that appear to fall outside of the basic library of forms, which are not simply related to the growth and curvature of a hexagonal cylindrical embryo. Some of these (Fig. 1k, l) give the impression of being mutated, intergrown and composite versions of the basic library of forms (Fig. 1a–j). Point, line and planar defects (which are intrinsic to self-assembled mesophases) may contribute to the distortions that seem to be inherent in some of these forms. We note that the observed shapes and surface patterns result from the growth rather than the drying process, and that disturbance of a quiescent synthesis by agitation leads to similar, but broken, forms of the mesoporous silica morphologies.

Representative polarizing optical microscopy (POM) images for rope and discoid mesoporous silica morphologies are shown in Fig. 3. A survey reveals that they are optically anisotropic and show evidence for a common kind of optical birefringence pattern. When the discoid and related morphologies are observed with the incident light along their topologically unique rotation axis, one observes a direction image characteristic of an uniaxial system viewed along the optic axis¹⁸. This image can be described as a roughly symmetrical black cross (the isogyres) with the arms parallel to the directions of the crossed polars, a central point (the melatrope), and concentric coloured rings in the four quadrants (the isochromes). A similar image is seen for the toroids and related morphologies. By contrast, when the discoids and gyroids are observed orthogonally to the topologically unique rotation axis (which is itself aligned with the directions of the crossed polars), a broad diffuse cross is observed (the isogyres) that only occupies the centre of the field over a few degrees of rotation off axis.

There exist four possible explanations for these POM images: (1) a high degree of order from the surfactant headgroups adjacent to disordered silica walls of the mesochannels; (2) strain-induced anisotropy from the amorphous silica walls of the mesostructure; (3) a high degree of regularity of the tetrahedral $\text{SiO}_4/\text{SiO}_3\text{OH}$ building blocks that constitute the silica walls of the mesochannels; and (4) the mesoscale periodicity of the channels. The first three possibilities can be reasonably eliminated based on experimental observations that the POM images remain unchanged on removing the surfactant-templating, and on annealing the template-free mesoporous silica morphologies at 540 °C, and that ^{29}Si magic-angle spinning (MAS) NMR spectra of mesoporous silica samples show a Q_3/Q_4 ratio of ~0.6, which is not significantly different from those previously reported for mesoporous silicas (^{29}Si solid-state NMR spectra were recorded on a Bruker DSX 200 MHz spectrometer at 40 MHz using a 90° pulse with a delay time of 600 s)¹⁹. Therefore, the observed optical anisotropy arises from the global polarization of an average electron density distributed around a hexagonal periodic array of mesopores. In other words, the optical birefringence is associated with the spatial anisotropy of the air–silica interface within the channels.

Some insight into the growth model has been obtained by examining high-resolution SEM and TEM images (Fig. 4). The 1,000 Å-wide rope shown in Fig. 4a is thin enough to be imaged directly using a high-voltage electron beam without having to resort to microtome sectioning. The parallel curving lines with a spacing of ~25 Å, which run the entire body length of the rope, is exactly one-half of the channel centre-to-centre distance. This is expected when the hexagonal mesostructure is viewed along the (210) direction of the rope¹³. It is only feasible to obtain such a clear image of the internal channel plans of the rope if there exists almost perfect alignment of the channels throughout the body of the rope. Noteworthy in the TEM image is the pronounced surface irregularity, predominantly located in the basal plane of the head of the rope (Fig. 4a). It is likely that this structure portrays growth instabilities, rigidified in silica, of the accretion and polymerization

of surfactant–silicate micelles by the evolving hexagonal cylinder. The roughness of this growth front revealed by TEM is consistent with that estimated from SEM images of similar regions of the rope, and is noticeably larger for the head (~300 Å) than the body (~100 Å) regions (Fig. 4b). This observation concurs with the idea that the deposition process is favoured at the sticky surfactant–silicate ends of the evolving hexagonal cylinder^{15,16}.

The unusual forms exhibited by these mesoporous silica morphologies appear to be unrelated to the faceted and geometric polyhedra of regular crystals grown under equilibrium conditions²⁰. Although these mesoporous silica forms are complex and have natural form, their geometries are not random but instead reveal an underlying growth pattern characteristic of a system that is far from equilibrium and not necessarily described by a free-energy function²¹. The growth process may well be nonlinear and under diffusion–reaction control, and thus (depending on the choice of reaction parameters) could lead to either smooth, abrupt or periodic alterations in the growth direction of the embryo, manifest as the observed arc and crankshaft rope forms, having smooth or faceted surfaces.

In this model, a hexagonal cylindrical surfactant–silicate embryo^{15,16} undergoes polymerization and growth through the accretion of surfactant–silicate micelles. The diversity of mesoporous silica morphologies reflects an underlying order based on the growth and curvature of this hexagonal cylinder. The geometrical shapes and surface patterns that emerge from this scheme represent the morphogenesis of mesoporous silica that has been frozen along the reaction coordinate in space and time. It is evident that supramolecular templating is able to create inorganic materials with natural form^{1,14}. □

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Conformational identification of individual adsorbed molecules with the STM

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The structure and conformation of a molecule determine its chemical and physical properties. Molecular conformation at interfaces is of particular importance in organic thin films¹: in organic optoelectronic devices^{2,3}, for example, charge carrier injection is influenced by interfacial properties⁴. Here we present a real-space conformational analysis of individual porphyrin molecules using scanning tunnelling microscopy^{5,6}. Porphyrins have been used as model systems to study charge transfer⁷ and *in vivo* photoactivation of drug precursors⁸, and have also been used in organic light-emitting diodes⁹. We find that changes in the porphyrins' conformation occur predominantly by rotations around the bonds to four tertiary butyl appendages, which differ on different metal substrates. On corrugated gold(110) surfaces, we identify two different conformations as the precursory (metastable) and final states of adsorption. This kind of conformational adaptation to a surface may be general for adsorbed organic molecules, and might have important consequences for the technological applications of organic thin films.

Cu-tetra[3,5 di-*t*-butylphenyl]porphyrin (Cu-TBPP) belongs to a class of porphyrins with four phenyl-based substituents of symmetrically bound, interconnecting carbon atoms on pyrrole rings (Fig. 1a). X-ray diffraction has shown that tetraphenylporphyrin (TPP) derivatives exhibit dihedral angles of 60–90° between the porphyrin ring and the phenyl substituents in bulk crystal samples, depending on the nature of the substitution and their local environment^{10–12}, with specific radical forms exhibiting rotations as low as 40°. The overall conformation of TPP derivatives can be ascribed to a steric repulsion between the *ortho*-substituents on the phenyl ring and the outermost substituents on the pyrrole ring. Together with the competing effect of the degree of π overlap with the porphyrin ring, this repulsion determines the rotation of the phenyl groups. Figure 1b illustrates the nature of steric repulsion on Cu-TBPP on a Cu(100) surface. The rotation angle around each of the four phenyl–porphyrin bonds is the predominant conformational factor that determines the shape of Cu-TBPP.

Atomically clean, single-crystal metal surfaces (Cu(100), Au(110), Ag(110)) were used as substrates and prepared by cycles of Ne⁺ sputtering and annealing at 700 K. A fraction of a monolayer of Cu-TBPP was sublimed in ultra-high vacuum, equilibrated by a subsequent anneal, and characterized *in situ* by a scanning tunnelling microscope (STM). STM images of Cu-TBPP in its adsorbed state consist of four bright lobes¹³. Both their dimensions and theoretical STM electron-scattering quantum-chemistry calculations^{14–16} confirm that the lobes correspond to di-*t*-butylphenyl (DBP) ligands¹³. The porphyrin core does not contribute significantly to the image because it is supported ~0.7 nm above the surface and is consequently electronically decoupled from the substrate. Figure 2 shows STM topographs of Cu-TBPP molecules adsorbed on a different surface, Au(110), after deposition in ultra-high vacuum in three stages of annealing. Three characteristic shapes of adsorbed Cu-TBPP can be identified in Fig. 2a from the change in geometry of the four lobes corresponding to one

molecule. The distribution of the observed molecular geometries as a function of annealing duration and temperature (Fig. 2a–c) permits assignment of mobile precursor states (P), final adsorption states (F) as well as molecules immobilized at kinks and steps (X).

Our determination of single-molecule conformation relies on the shape of the four quadrilateral lobes at each molecule, defined by their aspect ratio, which varies from ~1.4:1 for the state on top of the Au-2 × 1 terraces (P in Fig. 2) to 1:1.6 for the final states (F in Fig. 2) in the areas where the Au atoms are observed to form a 6 × 1 reconstruction. This process is facilitated by the small energy difference of ~2 meV between 1 × 2, 1 × 3 and 1 × 4 phases of Au(110) (ref. 17). The molecular aspect ratios reflect different conformational forms of Cu-TBPP molecules. The relative position of the uppermost *t*-butyl group corresponding to each lobe of the molecule is determined by the rotation angle for the phenyl–porphyrin bond of each DBP substituent. From the aspect ratio of the four lobes in a single molecule we can determine its conformation. The STM images are consistent with antisymmetric rotation of two opposite DBP substituents.

Figure 3 compares high-resolution STM data of adsorbed Cu-TBPP molecules on Cu(100), Au(110) and Ag(110) substrates. Here the dimensions and symmetry of the lobes in the STM images are observed to differ considerably and were used to analyse the bond angles of the four DBP groups with respect to the porphyrin ring. Molecular models were constructed with bond angles chosen to match the experimentally observable dimensions. These models were then used to determine the interaction of the molecule with the substrate. When adsorbed on a Cu(100) surface the molecule retains

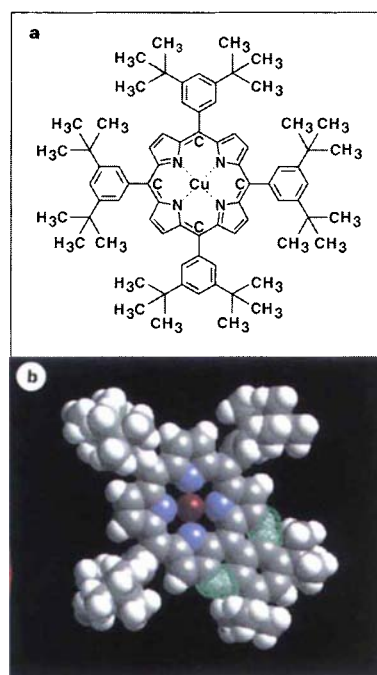


Figure 1 **a**, Structure of Cu-tetra[3,5 di-*t*-butylphenyl]porphyrin (Cu-TBPP). **b**, Space-filling representation of the conformational flexibility of Cu-TBPP. Colours correspond to the elements in **a** as follows: H, white; C, black; N, blue; Cu, red; two pairs of sterically interacting hydrogen electron shells are shown in green for the planar conformation of the substituent at bottom right. Consequently, the di-*t*-butylphenyl (DBP) substituents are oriented out of plane with the porphyrin in the minimum energy conformation. This orientation is displayed for the three remaining substituents. Owing to the weak overlap of the π states at this bond angle, the porphyrin–phenyl bond of TBPP is predominantly a single (σ) bond that does not participate in the conjugated electron system.

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a conformation close to minimum energy for Cu-TBPP in vacuum, with all four DBP groups rotated $\sim 90^\circ$ with respect to the porphyrin plane. This results in the square pattern observed with the surface. It has the same symmetry as the face-centred-cubic (100) substrate, but is rotated 20.5° with respect to the [010] direction. Electron-scattering quantum-chemistry calculations also support this perpendicular orientation of the DBP substituents (H. Tang and C. Joachim, personal communication). In contrast,

two distinct rotations were determined for Cu-TBPP adsorbed on the Au(110) surface (Fig. 3b and c) corresponding to an anti-symmetric tilt of two opposite side groups by 65° and 45° , respectively. The 65° form is predominant after short (<10 min) annealing cycles, whereas the 45° form is predominant after longer annealing cycles. Consequently we assign the 65° conformation to a metastable precursor state and the 45° form to the thermodynamically stable conformation. The most pronounced

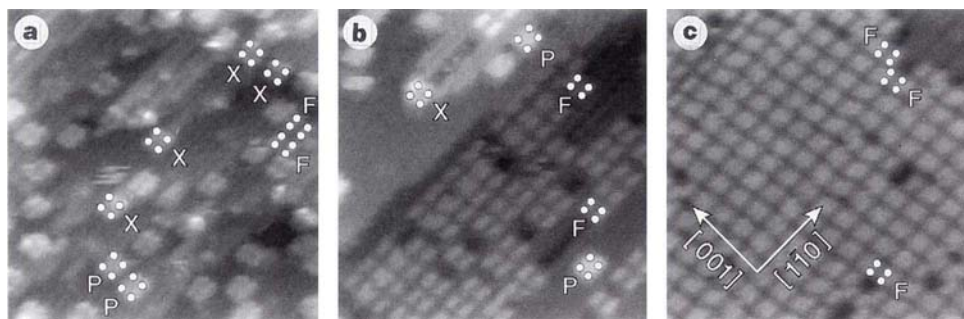


Figure 2 Three stages of an extended annealing study of Cu-TBPP on Au(110). All samples were started by deposition of a fraction of a monolayer (20–30%) of Cu-TBPP on Au(110) at $\sim 150^\circ\text{C}$. Individual molecules are identified as four protrusions of quadrilateral shape: these are shown in white and labelled. **a**, After annealing for 3 min at $\sim 200^\circ\text{C}$, randomly distributed molecular species can be identified by different adsorption sites and shapes. (P, on top of 2×1 terrace with rectangular shape and aspect ratio $\sim 1.4:1$; X, at step and kink sites with approximately square symmetry; F, embedded in a 6×1 trench formed by removing two 2×1 adatom rows of rectangular symmetry with an aspect ratio

of $\sim 1:1.6$). **b**, After annealing for 9 min at slightly increased temperatures ($\sim 250^\circ\text{C}$), STM reveals extended rows of molecules in state F. The number distribution of states shifts towards the embedded adsorption state F. States P and F tend to nucleate on rows and terraces. **c**, Annealing for 12 min on a similar substrate reveals extended terraces covered with a monolayer of Cu-TBPP in the final state F. Extensive faceting/step-bunching of the vicinal surface has been reproducibly observed after long anneals of the co-adsorbed molecule. (Imaged area, $\sim 23 \times 23$ nm).

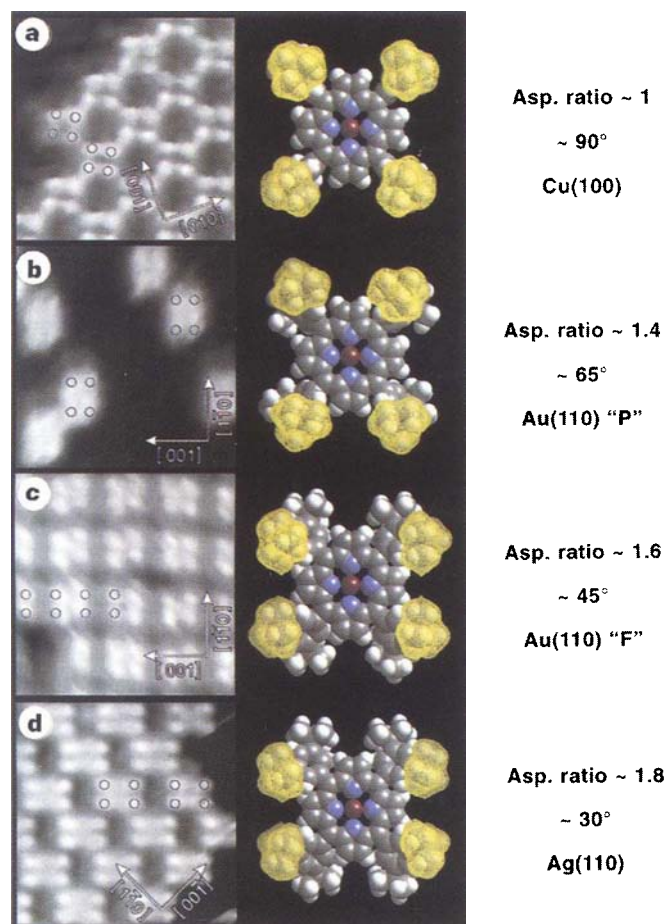


Figure 3 Conformational identification of Cu-TBPP in four stages. The four-lobed quadrilateral forms with varying aspect ratio are clearly visible in the STM data (left-hand column) of Cu-TBPP adsorbed on all these surfaces (imaged area, $\sim 10 \times 10$ nm). The lobes appear independent of variable chemical bonding with the substrates and can be identified as the uppermost *t*-butyl groups of each of the four large (~ 1 nm) DBP substituents. The slight deviations from the rectangular shape observed for Cu-TBPP on Au(110) are a consequence of molecular motion while scanning. In the models shown in the centre column, the four bond angles of the phenyl-porphyrin bonds of each molecule have been determined such that the conformation of the model corresponds to STM data. The experimentally determined aspect ratio and the resulting bond angle are listed in the right-hand column, together with substrate type and orientation. The uppermost *t*-butyl groups are shown in yellow to emphasize the molecular subunits visible in the STM data. **a**, Cu(100), square symmetry, $\sqrt{58} \times \sqrt{58}$ superstructure; 0° bond rotation. **b**, Au(110) mobile precursor state on 2×1 terraces; rectangular symmetry parallel to (110); $\pm 25^\circ$ bond rotation. **c**, Au(110) final state of adsorption after extended annealing; rectangular symmetry perpendicular to (110) rows after coordinative 6×1 reconstruction; $\pm 45^\circ$ bond rotation. **d**, Ag(110) maximum aspect ratio rectangle on unreconstructed 1×1 terraces; $\pm 60^\circ$ bond rotation. The observed difference in molecular corrugation of the centre porphyrin versus the substituents decreases from 0.8 nm to 0.3 nm with progressing conformational flexure **a** to **d**. This contrast change reflects shifts of the porphyrin groups' electronic states and their coupling to the substrate.

conformational change upon adsorption is observed for Cu-TBPP on Ag(110) (Fig. 3d), where the single adsorption state is characterized by a dihedral angle of 30°.

From this analysis we conclude that the molecular conformation of Cu-TBPP is driven by the nature of the molecule-surface interaction. This conformational adaptation within the molecules occurs in addition to the general tendency for an adsorbate molecular lattice to rotate with respect to the substrate in order to minimize the inequivalent number of adsorption sites¹⁸. It also goes beyond the observation of a gauche conformation in molecular layers¹⁹. The saturated hydrocarbon group on the DBP substituents interacts with the surface by a weak chemisorption and permits molecular mobility¹³. Small deviations of the Cu-TBPP conformation ($\sim 90 \pm 10^\circ$) result from modification of the weak chemisorption in response to the atomic corrugation and spacing. In contrast, larger conformational adaptations ($\sim 45^\circ$ and more) are dominated by stronger π -metal interaction at close proximity (typically $\ll 0.5$ nm) of the delocalized electron system of the phenyl and the porphyrin components to the metal.

Conformational analysis of adsorbed molecules permits a semi-quantitative analysis of the adsorbate molecular interaction energy. The degree of rotation of the phenyl-porphyrin bonds balances the intramolecular steric hindrance with the molecule-surface interactions. The rotational barrier for one phenyl-porphyrin bond of tetra-aryl porphyrins in liquids has been measured by thermally activated isomerization of specific isomeric forms (isolated atropisomers) using NMR spectroscopy²⁰⁻²² and chromatography. Depending on the central metal atom, substituent and solvent, values of 80–140 kJ per mol per bond²⁰⁻²² have been published. This compares favourably with the 100 kJ per mol per bond resulting from a numerical simulation performed for a Cu-TBPP molecule in vacuum¹⁷. For the 30° dihedral angle observed for Cu-TBPP adsorbed on Ag(110), we estimate an energy of about 320 kJ per mol per bond. This conformation change is accompanied by an increased mixing of the π orbitals of the porphyrin and the phenyls, resulting in reconjugation of π orbitals throughout the molecule. As a result the lateral dimension of the TBP lobes in the STM data for Ag(110) (Fig. 3d) increases, whereas the contrast difference between substituent and centre of the porphyrin decreases to 0.3 nm (compare Cu(100) ~ 0.8 nm). For comparison, we attempted to perform scanning tunnelling spectroscopy experiments on various parts of the adsorbed porphyrins; but our current-voltage measurements resulted in decay of the molecules, precluding conventional analysis.

STM recognition of the orientation of molecular subunits should provide insights into the detailed geometric factors involved in adsorbent-adsorbate interactions. The conformational changes that we observe reflect the interaction strength of the molecule with the substrate and its geometry. □

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Increased plant growth in the northern high latitudes from 1981 to 1991

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Variations in the amplitude and timing of the seasonal cycle of atmospheric CO₂ have shown an association with surface air temperature consistent with the hypothesis that warmer temperatures have promoted increases in plant growth during summer¹ and/or plant respiration during winter² in the northern high latitudes. Here we present evidence from satellite data that the photosynthetic activity of terrestrial vegetation increased from 1981 to 1991 in a manner that is suggestive of an increase in plant growth associated with a lengthening of the active growing season. The regions exhibiting the greatest increase lie between 45° N and 70° N, where marked warming has occurred in the spring time³ due to an early disappearance of snow⁴. The satellite data are concordant with an increase in the amplitude of the seasonal cycle of atmospheric carbon dioxide exceeding 20% since the early 1970s, and an advance of up to seven days in the timing of the drawdown of CO₂ in spring and early summer¹. Thus, both the satellite data and the CO₂ record indicate that the global carbon cycle has responded to interannual fluctuations in surface air temperature which, although small at the global scale, are regionally highly significant.

We have made use of data from the advanced Very High Resolution Radiometers (AVHRRs) on board the National Oceanic and Atmospheric Administration (NOAA) series of meteorological satellites (NOAA-7, -9 and -11). From daily observations of channel 1 (wavelengths ~ 0.58 – 0.68 μ m) and channel 2 (~ 0.72 – 1.1 μ m) reflectances, global land data sets of normalized difference vegetation index (NDVI) were produced^{5,6}. The NDVI is expressed on a scale from -1 to +1. It is between -0.2 and 0.05 for snow, inland water bodies, deserts and exposed soils, and increases from about 0.05 to 0.7 for progressively increasing amounts of green vegetation⁷. NDVI data are strongly correlated with the fraction of photosynthetically active radiation (wavelength 0.4–0.7 μ m) absorbed by vegetation⁸, that is, to the photosynthetic activity of vegetation canopies⁹. Two global data sets of NDVI were analysed: (1) the land segment of the joint NOAA/NASA Earth Observing System AVHRR Pathfinder data set at 8 km spatial resolution and 10-day intervals, for the period July 1981 until the end of June 1991⁶, and (2) the Global Inventory Monitoring and Modelling Studies

(GIMMS) AVHRR NDVI data set at a similar spatial resolution, but at 15-day intervals, for the period January 1982 until the end of December 1990¹⁰. The Pathfinder data were calibrated to correct for post-launch degradation from estimates of the relative annual degradation rates (in %) of the two channels: 3.6 and 4.3 (NOAA-7), 5.9 and 3.5 (NOAA-9) and 1.2 and 2.0 (NOAA-11)¹¹. NOAA-9 data were used for inter-satellite normalization. The GIMMS data were independently calibrated¹²; they are considered here to illustrate how a different calibrating scheme affects trends in the AVHRR data.

For each equal-area pixel and at either 10- or 15-day intervals, depending on which of the two satellite data sets was used, the maximum of NDVI with minimal atmospheric effects was retained¹³. The NDVI data from high northern latitudes ($>40^{\circ}\text{N}$) did not show anomalies related to the El Chichon volcanic eruption during the mid-1982 to 1983 time period. These effects in the low latitude data were not corrected for in either of the two satellite data sets.

The calibrated Pathfinder NDVI data still showed residual non-vegetation-related variations¹⁴. We revised them by adjusting the NDVI for a hyper-arid portion of the Sahara desert

($1.42 \times 10^6 \text{ km}^2$) which has been found to be invariant as viewed by all three satellites¹⁰. An alternate correction scheme based on desert pixels from 10°N to 50°N yielded nearly identical results. Importantly, when this desert correction was applied to the NDVI anomaly time series of desert pixels from five-degree latitude bands between 10°N and 50°N , the residuals resembled noise.

Time series of spatially averaged monthly NDVI, evaluated as the mean of three 10-day maximum value NDVI composites, comprising a 10-year record, are plotted, first directly for reference (Fig. 1a), and then as anomalies to display interannual variability (Fig. 1b). Averaged for regions north of 45°N (uppermost curve in Fig. 1b) the NDVI anomaly shows evidence of increasing amplitude, summer values being low early in the record, high near the end. The NDVI anomaly in the tropics shows a large increase starting from November 1988, which also coincided with the change in satellites from NOAA-9 to NOAA-11. A somewhat smaller increase is seen during the switch from NOAA-7 to NOAA-9 in January 1985, although this increase began in the last months of the NOAA-7 record (and the anomaly north of 45°N actually shows a decrease). This raises a question regarding anomalous variations in NDVI from sensor changes. Although efforts have been made to establish

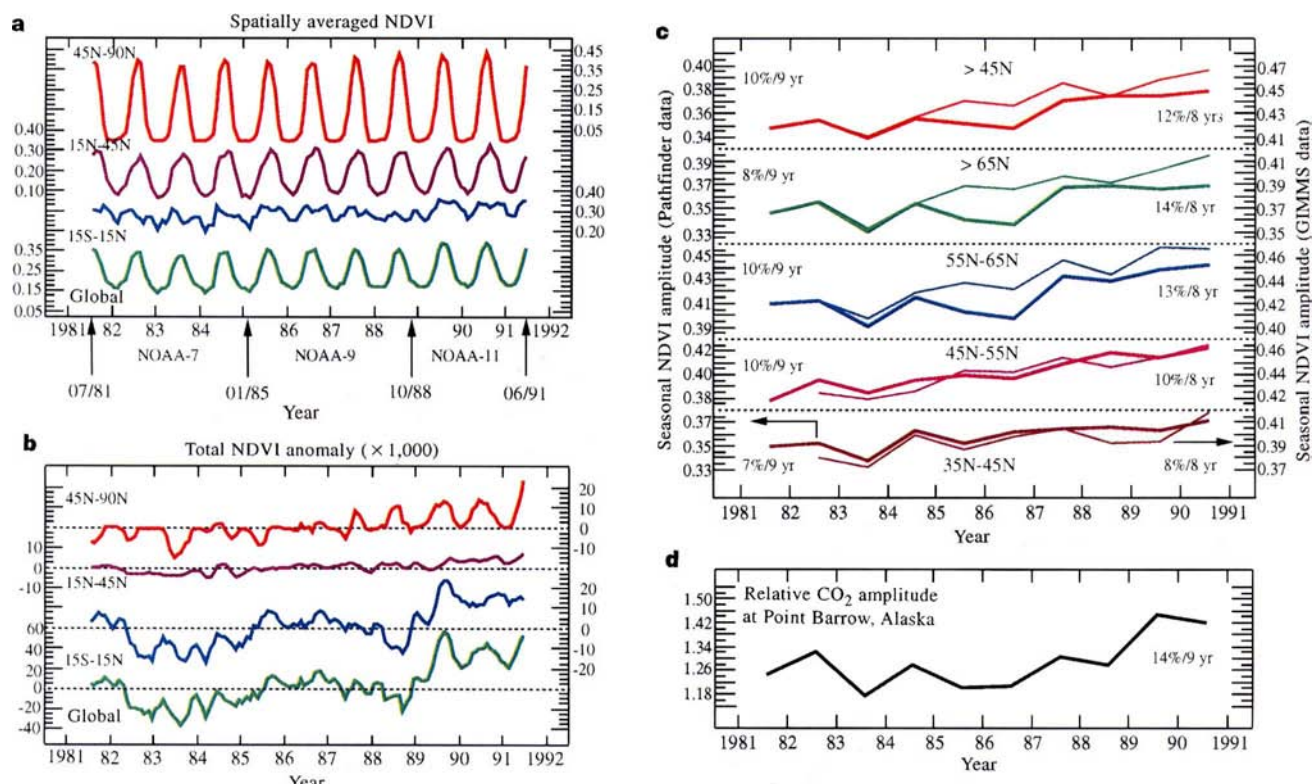


Figure 1 Time variations in normalized difference vegetation index (NDVI) compared with changes in amplitude of the seasonal cycle of atmospheric CO_2 for the period from July 1981 to the end of June 1991. The data in **a** and **b** have been smoothed by a 3-month running mean. Zonal total NDVI and its anomaly were calculated from pixels having a 10-year monthly average NDVI greater than 0.1 and within 3σ of the monthly average. The first condition guaranteed that bare or sparsely vegetated pixels were not included in spatial averages, while the second condition removed most of the influence of snow and bad scan-lines. **a**, Monthly average NDVI for selected latitudinal bands and the whole globe. The zonal total NDVI was normalized by the total vegetated land area in the month of August to obtain a zonal average that exhibited seasonality¹⁴. **b**, Monthly total anomalies of the above, expressed as departures from the 10-year record averages of monthly NDVI, summed over each latitudinal band¹⁴ for each month. The vertical scale of the global plot is twice that of individual latitudinal bands. **c**, Seasonal amplitude of NDVI averaged over selected latitudinal bands. The amplitude, defined as the July

and August average, is a good approximation because, at the northern latitudes shown, the winter-time NDVI value is close to zero. Spatial averaging was for July and August data combined over pixels with 10-year averages of NDVI greater than 0.1, in order to exclude bare areas, such as the great deserts of Asia. Results from both the Pathfinder (left ordinate) and GIMMS (right ordinate) NDVI data sets are shown together with the corresponding rates of increase. The higher rates of increase inferred from GIMMS data may be due to the lack of desert correction for the version of GIMMS data used in this analysis. **d**, Seasonal amplitude of atmospheric CO_2 relative to a base-period of 1961-67 as registered at Point Barrow, Alaska (71°N , 157°W)¹. Linear trend estimates of the increase in seasonal amplitudes of NDVI and CO_2 are statistically significant (10% level) for all latitudinal bands shown. However, the limitations of regression analysis on short samples, that is, the determination of trend in the presence of low-frequency variations, must be noted.

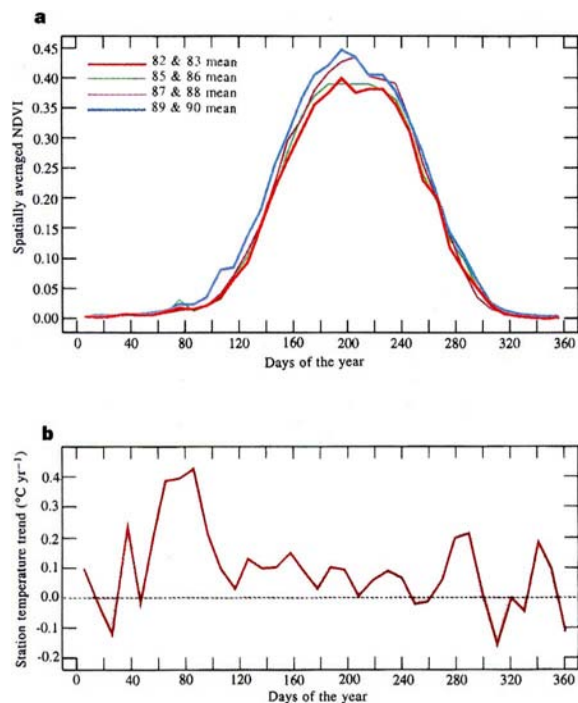


Figure 2 Interannual changes in seasonality of NDVI and in surface temperature, averaged north of 45° N, for selected pairs of years from 1982 to the end of 1990. **a**, NDVI averaged from 10-day maxima in NDVI for 1982–3, 1985–6, 1987–8 and 1989–90. Data from 1984 were not included because the number of years was odd. Spatial averaging was similar to that described in Fig. 1a legend. Changes in the timing of the active growing season over this 9-year record period were estimated from differences between the first and last bi-yearly average profiles at six threshold values of NDVI (from 0.1 to 0.35 in 0.05 increments). These values occurred during intervals of about 60 days each in spring and autumn when, respectively, NDVI was increasing and decreasing, at almost a constant rate. The six estimates each of the timing of rise and fall of NDVI may actually be correlated because of low-frequency variations (for example, soil moisture and/or equatorial sea surface temperature oscillations), and therefore, the standard errors given in the text must be interpreted in light of this limitation. We also inferred similar changes in the active growing season duration from an alternate pixel-by-pixel and year-by-year analysis¹⁴. **b**, Changes in the annual cycle of near-surface air temperature from 1982 to 1990. Daily thermometer observations of maximum and minimum temperature were averaged in order to approximate daily mean temperatures and interpolated on a 1 × 1 degree grid²⁷. The daily data were further averaged over three separate approximately 10-day periods per month to obtain 36 observations per year. These 10-day average temperatures were then linearly regressed on the year (from 1982 to the end of 1990) to obtain the slopes shown here.

proper inter-sensor calibration linkages^{11,12}, some residual effects cannot be ruled out, especially between NOAA-9 and NOAA-11¹⁴. This situation, for example, confounds proper interpretation of the tropical NDVI anomaly time series. For instance, intense sea surface temperature (SST) oscillatory events in the tropical Pacific and Atlantic oceans from 1982 to early 1989 have been linked to decreased vegetation growth in large regions of the semi-arid tropics¹⁵. The increase in tropical and global NDVI anomaly starting from late 1988 also coincided with an unprecedented decline in atmospheric CO₂ anomaly, from a peak value in late 1988 to a minimum in late 1993¹⁶. Nevertheless, these interpretations, as they involve the NDVI data, are limited by possible sensor change effects.

Changes in the amplitude of the seasonal cycle of NDVI at northerly latitudes greater than 35° N are plotted in Fig. 1c, as characterized by changes in the July and August average NDVI. This broad measure of the seasonal maximum approximates the seasonal amplitude because winter-time NDVI at these northern latitudes is close to zero (compare Fig. 1a). The seasonal amplitude, by this definition, increased by 7 to 14%, depending on the latitude and data set, from 1981 or 1982 to the end of 1990 (Fig. 1c). Because NDVI is a measure of photosynthetic activity of vegetation as noted above^{8,9}, this increase indicates a substantial change in photosynthetic activity of plants at higher northern latitudes. A similar increase (14%) is indicated in the amplitude of the seasonal cycle of atmospheric CO₂ measured at Point Barrow, Alaska¹ (Fig. 1d). This CO₂ cycle, although observed in the Arctic (71° N), registers changes in CO₂ gas exchanges, and hence in the biotic activity of plants and soil over all northern temperate and polar latitudes¹⁷. Together, the NDVI and CO₂ data indicate increased biospheric activity north of about 35° N. Two recent studies have also reported increased photosynthetic activity in the northern high latitudes as increased biomass from deposition in European forests¹⁸ and from tree-ring analysis in Mongolia¹⁹, respectively.

Timing of the seasonal rise and fall in NDVI suggests possible changes in the length of the active growing season, that is, the period during which photosynthesis actually occurs (as opposed to the concept of growing season, measured for example in degree days. As shown in Fig. 2a in Pathfinder data, the rise in NDVI, spatially averaged from 45° N to the northern limit of the data, came progressively earlier in the season between 1982 and 1990, as

shown by successive 10-day averages, where each plot shows an average over two years for clarity. Because spatially averaged NDVI rose each year at nearly a constant rate from early April (about day 110) to late June (about day 170) the advance in the active growing season is apparent, notwithstanding the relatively coarse time resolution (10 day) afforded by the NDVI data. From six estimates of the time advance at six successive thresholds of NDVI, we estimate an advance of 8 ± 3 days (Fig. 2a).

An advance of about 7 days in the seasonal cycle was previously inferred from atmospheric CO₂ data as having taken place between the 1960s and early 1990s, with most of the increase occurring after 1980 (Fig. 1 of ref. 1). The NDVI data suggest that this increase occurred over an extensive region of the extratropical Northern Hemisphere. The NDVI data in Fig. 2a further indicate a prolongation of the declining phase of the active growing season, estimated at 4 ± 2 days between 1982–3 and 1989–90. Therefore, the active growing season north of 45° N appears to have lengthened by 12 ± 4 days over the 1980s. These estimates must be interpreted as suggestive of a longer active growing season, rather than in an absolute sense, in view of the coarse temporal resolution (10 days) and residual atmospheric effects in NDVI data. The associated standard errors given here are not rigorous, for low-frequency variations in NDVI data invalidate the assumption of statistical independence required of the successive threshold values.

Variations in the amplitude and timing of the seasonal cycle of atmospheric CO₂ have shown an association with surface air temperature consistent with the hypothesis that warmer temperatures have promoted increases in biospheric activity outside the tropics^{1,2}. A likely cause is an increase in the length of the active growing season brought about by warmer temperatures¹. As shown in Fig. 2b, a pronounced increase in late-winter and early-spring temperatures took place over the period of NDVI changes, especially during March.

Because of their high spatial resolution (relative to ground-based meteorological measurements), NDVI data provide spatial detail of where the average changes in amplitude and timing of the active growing season occurred. To address regional variations in NDVI, we show in Fig. 3 a map related to the time plots shown in Fig. 1 together with a map of the 9-year average of NDVI for comparison.

The linear rate of change in NDVI, averaged over the 9 years of

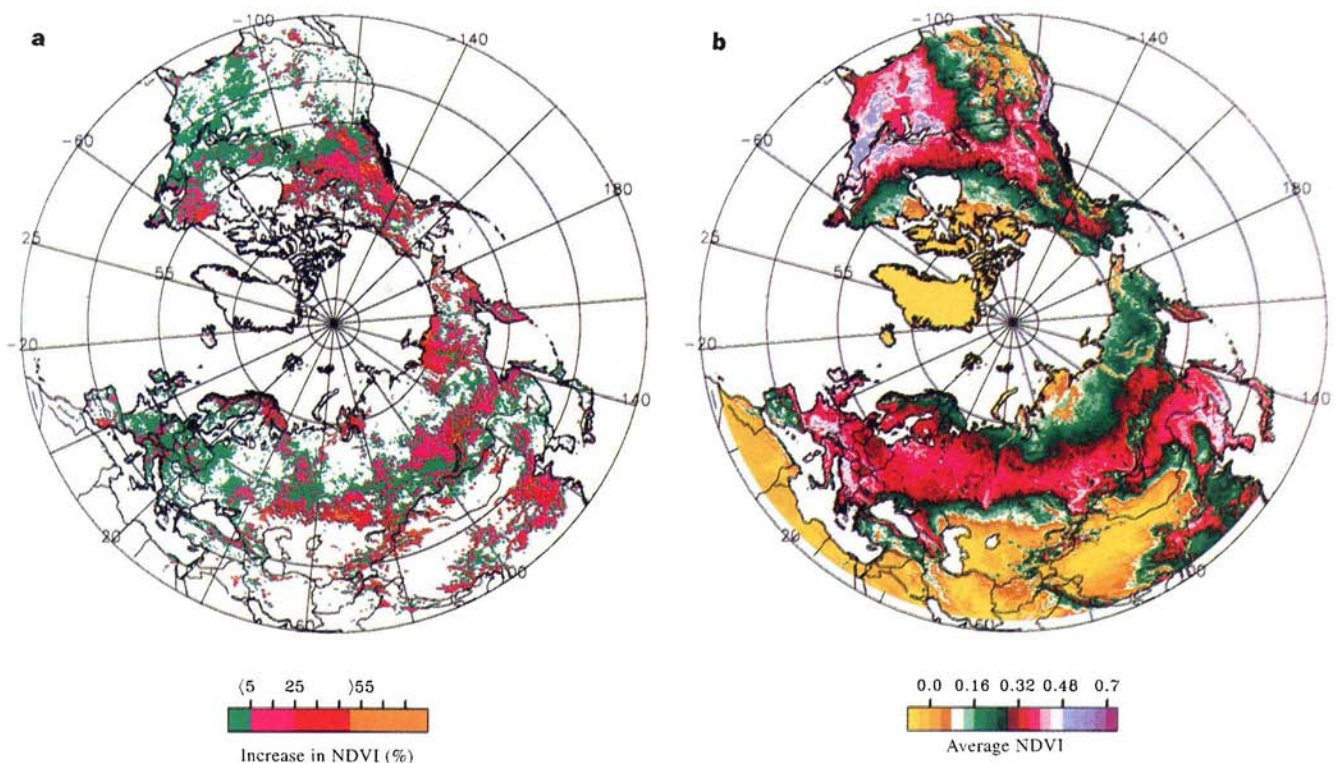


Figure 3 Geographical distribution of the change from 1982 to 1990 in NDVI of land areas north of 27.75°N, expressed as the average over the northern active growing season of May to the end of September. **a**, NDVI increase in percentage over 9 years, determined by linear regression of year-to-year northern growing season averaged NDVI, aggregated to 0.25×0.25 degree pixels from original 8-km-resolution data. Only estimates with a positive slope and a statistically

significant (10% level) regression coefficient were contoured. There were no pixels with statistically significant negative slopes. Again, we emphasize the limitations of regression analysis on short-term samples. **b**, Nine-year average NDVI over northern active growing season of May to the end of September determined by averaging the monthly NDVI of 0.25×0.25 degree pixels from 1982 to 1990.

seasonal NDVI data in northern latitudes, from 1982 to the end of 1990, are mapped in Fig. 3a. Data were averaged from May to the end of September, to approximate the main active growing season of land vegetation in the Northern Hemisphere.

In Eurasia, a band of increasing NDVI extends from Spain in a northeasterly direction across Asia to the western Pacific Ocean. In this band, central Europe, southern Russia, and a broad region near Lake Baikal in Siberia are most affected. Outside this band, northern Scandinavia, northern China, and northeastern Siberia are also strongly affected. In North America, a band of increasing NDVI extends from Alaska in a southeasterly direction to the Great Lakes, thence northeasterly to Labrador. In this band, northwestern Canada is most strongly affected. Outside this band, the continental United States (excluding Alaska) and the area around the Hudson Bay show little change in NDVI.

In general, the regions of greatest increase in NDVI are inland from the oceans, except in the Arctic, and are north of 50°N. The prominent bands of increased NDVI referred to above in both Eurasia and North America, correspond generally to areas of high NDVI (Fig. 3b). Thus most of the areas where changes in NDVI amplitude and seasonality were observed are also regions of significant vegetation density. Notable exceptions are several Arctic regions in Eurasia where NDVI rose sharply from low initial values.

We believe the increasing trend in photosynthetic activity of the northern high latitudes, inferred from satellite observations of NDVI amplitude and phase, to be robust despite varying satellite overpass times and the lack of an explicit atmospheric correction. These effects, however, could modify the magnitudes of NDVI amplitude and estimates of the active growing season duration.

Analyses of station temperature trends during 1961–90 indicate

pronounced warming over substantial areas in Alaska, northwestern Canada and northern Eurasia³. The greatest warming, up to 4°C, has occurred in winter. Only slightly less warming has occurred in the same regions in spring, but considerably less warming in summer and even less in autumn³. Associated with warming at high latitudes is an approximate 10% reduction in annual snow cover from 1973 to the end of 1992, especially an earlier disappearance of snow in spring (Table 1 of ref. 4). Where snow-lines have retreated earlier due to enhanced warming, we expect an early start of the active growing season.

The winter and spring warming in the interior of the continents of Asia and North America in the 1980s may be a result of natural causes not yet explained, but its timing is consistent with an enhanced greenhouse effect caused by build-up of infrared-absorbing gases in the atmosphere²⁰. The unusual warming which peaked near 1990 was of global extent. Although it amounted to a departure of only a few tenths of a degree from previous record temperatures²¹, it was associated with far greater warming in the spring months at high northern latitudes. Biospheric activity there, based on our analysis, increased remarkably as a result of this warming, suggesting that small changes in global temperature may reflect disproportionate responses at the regional level, and may be accompanied by positive feedbacks which can markedly influence processes such as photosynthesis and litter decomposition. □

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Large-scale tectonic deformation inferred from small earthquakes

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It is a long-standing question whether the focal mechanisms of small earthquakes can be used to provide information about tectonic deformation on a regional scale. Here we address this question by using a 28-year record of seismicity in the San Francisco Bay area to compare the strain released by small earthquakes with geological, geodetic and plate-tectonic measurements of deformation in this region. We show that on a small spatial scale, the strain released by small earthquakes is closely related to specific geological features. But when averaged over a regional scale, strain release more closely follows the regional pattern of tectonic deformation: this relationship holds for all but the largest earthquakes, indicating that the earthquake strain is self-similar^{1,2} over a broad range of earthquake magnitudes. The lack of self-similarity observed for the largest earthquakes suggests that the time interval studied is not large enough to sample a complete set of events—the fault with the highest probability³ for hosting one such missing event is the Hayward fault.

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Following the formulation by Kostrov⁴, the seismic strain of a block in the Earth's crust can be obtained from the geometric moment tensors

$$\epsilon_{ij} = \frac{1}{2V} \sum_{n=1}^N \mathcal{M}_{ij}^{(n)} \quad (1)$$

where $\mathcal{M}_{ij}^{(n)}$ is the geometric moment tensor of the n th earthquake, N is the number of earthquakes and V is the volume of the block. The geometric moment tensor of an earthquake is given by $\mathcal{M}_{ij} = \int_A u dA (u_i n_j + u_j n_i)$, where A is the surface of the fault, dA is a surface element, u is the slip in the earthquake, and u_i and n_i are the unit slip and fault-normal vectors, respectively. The geometric moment tensor $\mathcal{M}_{ij}$ referred to by Ben-Menahem and Singh⁵ as "potency", is related to the seismic moment tensor M_{ij} used in seismology by $M_{ij} = \mu \mathcal{M}_{ij}$ (ref. 6), where μ is the shear modulus.

The strain in a local region, $\epsilon_{ij}^{(loc)}$, is related to the strain in a larger region, $\epsilon_{ij}^{(reg)}$, by a fourth-order tensor that is a complicated function of time and space. Here we consider special cases in which they are assumed to be linearly related by

$$\epsilon_{ij}^{(loc)} = c \epsilon_{ij}^{(reg)} \quad (2)$$

where c is the amplitude of the local deformation, that is, the shape and the orientation of the local and regional strain tensors are identical but their amplitudes can be different. For homogeneous deformation, $c = 1$. If seismic strain is self-similar and if $\epsilon_{ij}^{(loc)}$ is the seismic strain by earthquakes in one scale, then equation (2) will apply at any scale with different values for c . Some examples are

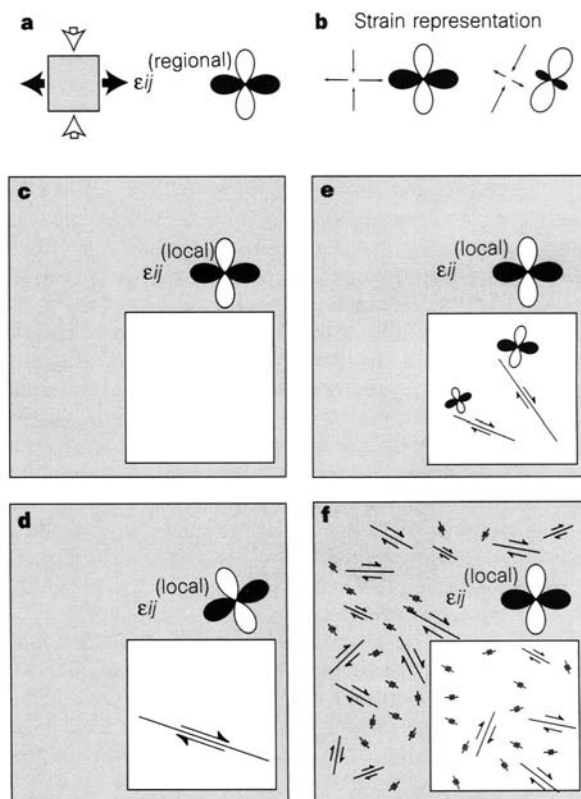


Figure 1 Homogeneous and inhomogeneous deformation in four different regions subject to pure shear. **a**, Boundary conditions and imposed strain. **b**, The strain tensor is represented by two-dimensional strain rosettes for the horizontal components. White lobes indicate directions of contraction, black lobes indicate directions of extensions. **c**, Homogeneous deformation. **d**, Inhomogeneous deformation due to an individual shear fault. **e**, Inhomogeneous deformation due to two faults. **f**, Quasi-homogeneous deformation due to many shear faults with a variety of orientations.

illustrated in Fig. 1. The (applied) regional strain is pure shear (Fig. 1a). We refer to the orientation and shape of a strain tensor as the strain pattern and use strain rosettes for the representation (Fig. 1b). In the absence of faults the strain is homogeneous and the strain pattern in a local region is identical to the regional strain (Fig. 1c). The strain due to slip on a single fault is in general different from the regional strain (Fig. 1d). Two shear faults are, in principle, sufficient to accommodate any regional shear although strain concentrations can be large (Fig. 1e). The strain pattern in a region that contains

many faults with a variety of orientations and homogeneous spatial distribution tends to equal the regional strain (Fig. 1f). Note that Fig. 1f is identical to Fig. 1c if the faults are small compared to the dimension of the region.

The larger San Francisco Bay area (Fig. 3a) is part of the plate boundary zone between the Pacific and North American plates. The regional deformation is primarily simple shear. South of 37°N nearly all the transform motion is accommodated on the San Andreas fault and the motion is mainly aseismic (creeping section).

Table 1 Seismic deformation in earthquake regions

Region	Direction of principal contraction	$\frac{\text{principal extensional}}{\text{principal contraction}}$	Number of events summed
Golden Gate	N3°W	2.9	58
San Francisco Peninsula	N31°W	0.29	170
Loma Prieta	N16°W	0.57	885
Hollister	N3°W	0.91	7,117
Calaveras fault	N14°W	0.99	2,303
Hayward fault	N12°W	0.88	341
Livermore	N33°W	1	1,239
San Gregorio fault	N20°W	0.59	75
North Bay	N28°W	0.77	98
Diablo Range	N26°W	0.77	182

Shown here are the directions of principal contraction and ratios between the principal components of the horizontal strain in the regions from Fig. 3, for all events with magnitude ≤ 3.33 .

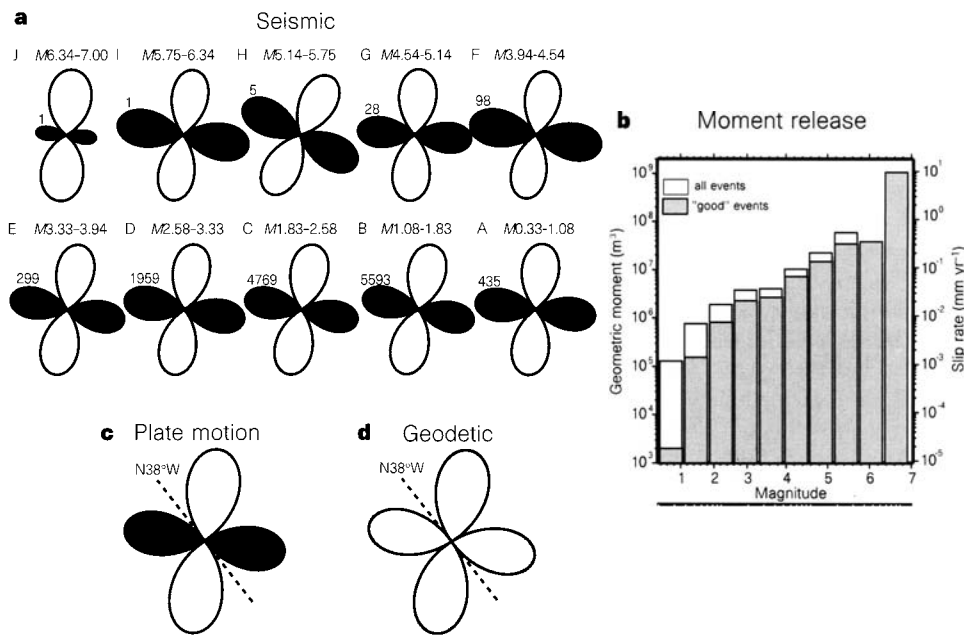


Figure 2 **a**, Two-dimensional strain rosettes representing the horizontal seismic strain patterns due to the earthquakes in Fig. 3 in magnitude ranges for which mean fault dimensions differ by factors of 2 (A–J). The numbers of events summed are shown next to the strain rosettes. The fault plane solutions are calculated from the first motion polarities using the computer program FPFIT³². The geometric moments are calculated for the local or duration magnitude, M , using the empirical relations $\log M_0 = 1.5M - 1.5$ for $M > 3.33$ and $\log M_0 = 1.2M - 0.5$ for $M \leq 3.33$ (modified from Bakun's³³ relations for the seismic moment using $\mu = 30$ GPa). In this study only "good" events satisfying some quality criteria are used; with uncertainties in strike, dip and rake less than 30°, 45° and 45°, and with magnitude ≥ 0.3 , depth ≥ 2 km, r.m.s. ≤ 0.3 s; horizontal location error ≤ 2.5 km; vertical location error ≤ 5 km; number of first motions ≥ 30 ; azimuthal gap $\leq 180^\circ$ (see ref. 32 for explanation of these parameters)

$\% \text{tp} \leq 0.4$; $\text{sdr} \geq 0.4$; $\text{fit} \leq 0.4$. **b**, Summed geometric moment within the magnitude intervals. The fault length is for circular faults and displacement-to-length ratios of 3×10^{-4} . The abscissa is not linear in magnitude because of two different moment-magnitude relations. The slip rate contribution is averaged over the observational period of 28 years and assumes that the events fall on a 250-km-long and 15-km-deep plate boundary fault. **c**, Predicted strain pattern for an orientation of the Pacific–North American plate boundary of N38°W, and relative motion of the Pacific plate in N34°W. **d**, Mean geodetic strain pattern from trilateration data¹⁶. The contributions of the San Francisco Bay network and of the Monterey network are summed. It is assumed that they cover three-quarters and one-quarter of the region studied, respectively. The strain rosettes in **c** and **d** represent 5% contraction perpendicular to N38°W.

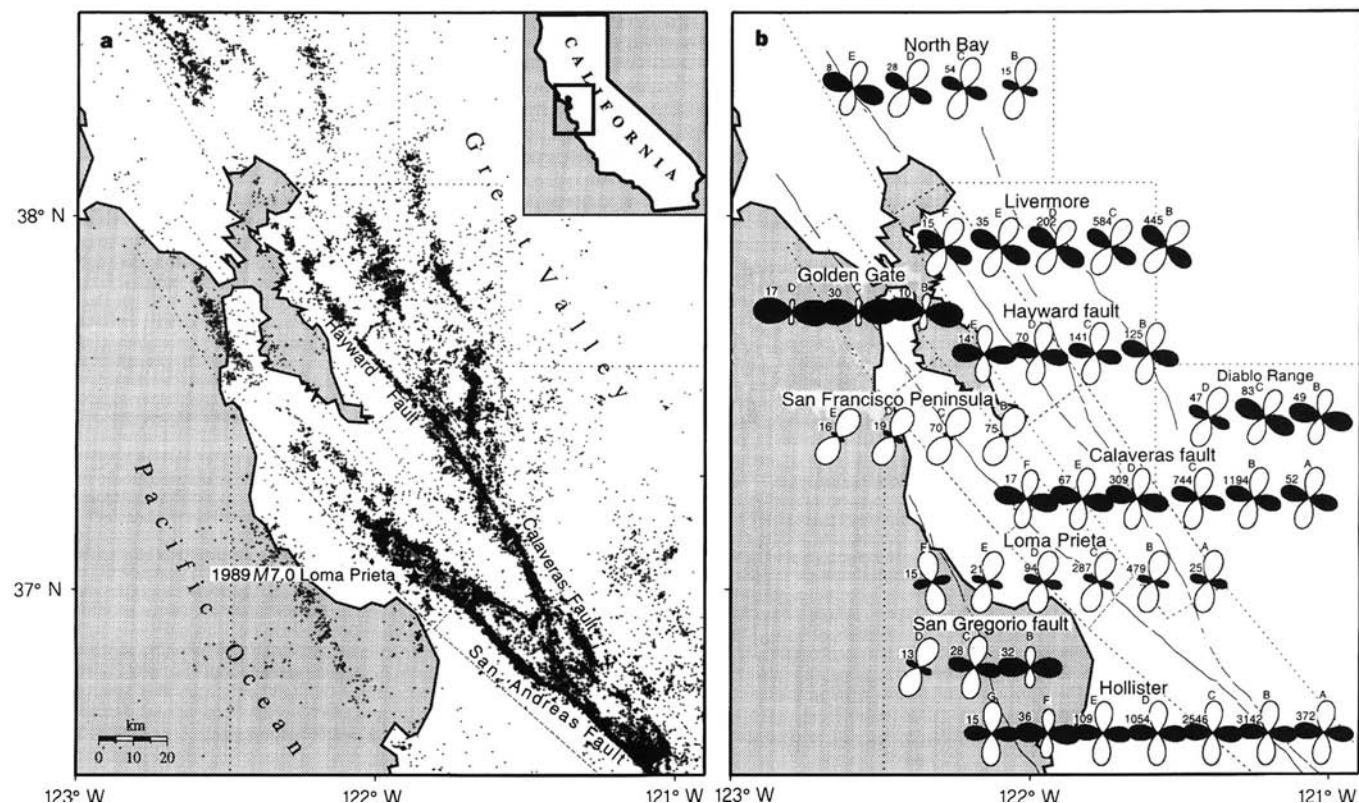


Figure 3 **a**, Location map showing the seismicity ($M > 1.0$) registered by the Northern Californian Seismic Network (NCSN) between January 1968 and December 1995 in the broader San Francisco Bay area. This study uses 15% of the 95,000 registered events with well constrained moment tensors. **b**, Two-

dimensional strain rosettes representing the horizontal strain due to earthquakes in the magnitude ranges defined in Fig. 2a (denoted by letters A–G). Only strain rosettes resulting from summing >5 events are shown. The numbers of events summed are shown next to the strain rosettes.

Further north, two-thirds of the deformation⁷ occurs on the San Andreas fault in occasional large earthquakes such as the 400-km-long rupture in 1906 or in smaller earthquakes that rupture fault segments 10–40 km long³. The remaining deformation is accommodated on subparallel strike-slip faults. The Hayward fault, for example, has been the source of two earthquakes with magnitudes (M) between 6.5 and 7 in 1836 and 1868 (ref. 8), and the Calaveras fault has ruptured in a sequence of earthquakes with $M > 5.5$ beginning in 1979; the $M = 6.2$ 1984 Morgan Hill earthquake was the largest^{9,10}. The recurrence times of 1906-type San Francisco earthquakes, of events on the Hayward fault and the Calaveras fault earthquakes are of the order of 250 years, 150 years and 80 years, respectively³. The seismic data covers a 28-year time period, which is nearly one-third of the shortest seismic cycle.

The seismic deformation of the San Francisco Bay area for different magnitude intervals is shown in Fig. 2a. The geometric moment tensors are derived from the fault plane solution and the magnitude for each event. The magnitude ranges have been chosen such that the seismic moments vary from one range to the other by a factor of 8. For events that are individually self-similar this corresponds to a factor of 2 change in mean fault dimensions between ranges^{11–13}. It can be seen that the strain patterns in the magnitude intervals below magnitude 3.94 are similar and can be interpreted as right-lateral shear across $N38^\circ W$ and 5% of shortening in perpendicular direction. The strain patterns differ only for the largest earthquakes where few events have been summed. The similarity of the strain patterns, together with the fact that the events occurred in different parts of the Bay area on faults with different orientations, suggests that the small faults slipped such that the ensemble resulted in a homogeneous reduction of elastic strain. If that is true, equation (2) is satisfied and the strain accommodated by small earthquakes

represents the applied deformation. The similarity of the strain patterns also indicates the self-similarity of earthquake strain. As many other aspects of rupture of rock are self-similar phenomena, such a self-similarity is expected to occur. Although the strain patterns for different magnitude ranges are similar, the strain amplitudes vary strongly. This can be seen in Fig. 2b where the summed release of geometric moment is shown. The nearly linear increase of the summed moment with the fault length is characteristic for tectonic environments with a b -value (the slope in the Gutenberg–Richter frequency of earthquake-occurrence relation) of ~ 1.0 (refs 14, 15). Note that the summed moment released by all the earthquakes and that by the ‘good’ earthquakes (with source parameters satisfying some quality criteria) are similar except in the two lowest magnitude intervals. The appropriateness of the quality criteria has been checked by examining the stability of all the seismic strain patterns obtained in this study using more and less strict criteria.

The seismic strain pattern can be compared with the strain pattern from plate motion and from geodetic¹⁶ information (Fig. 2c, d). The plate motion strain pattern is calculated from the relative velocity between the plates and the plate boundary direction, along which linear deformation is assumed not to occur¹⁷. According to plate motion models, the Pacific plate at the latitude of San Francisco moves $N34^\circ W$ with respect to North America^{18,19}. For a plate boundary direction of $N34^\circ W$, the San Francisco Bay area would shear right-laterally at $N34^\circ W$. The average strike of the major strike-slip faults, however, is more westerly. For a plate boundary direction of $N38^\circ W$ the predicted mean deformation contains some boundary normal contraction.

The good agreement between the seismic strain patterns in the lower magnitude intervals ($M = 0.33$ – 3.94), and the strain patterns

from the plate motion and from geodesy clearly shows that the deformation by the small earthquakes is in direction identical to the applied deformation, that is, the seismic (local) deformation and the applied (regional) deformation are linearly related by equation (2). The condition for this to be true appears to be that a sufficient number of earthquakes with comparable size and on faults with a variety of orientations are summed. If not, the deformation will be different because some event types are missing. This is the case for the higher magnitude intervals. In the $M = 3.94\text{--}6.34$ range, some earthquakes with a significant reverse component are absent. In the $M = 6.34\text{--}7.00$ range, a strike-slip event is missing. The fault with the highest probability³ for such an earthquake is the Hayward fault.

We note at this stage that the seismic strain pattern, the predicted strain pattern, as well as the geodetic strain pattern imply that, in the San Francisco Bay area, a shortening of $\sim 5\%$ of the shear deformation (2 mm yr^{-1}) occurs normal to the plate boundary (assumed to be oriented $N38^\circ W$). This is indicated by various geological and geophysical evidence^{20–23}, but has been questioned because the geodetic data has been interpreted differently^{16,24}. (Those studies examined the geodetic data for shortening perpendicular to the plate motion direction ($N34^\circ W$) and not perpendicular to the plate boundary direction ($N38^\circ W$).)

So far this study has been concerned with the overall deformation across the entire San Francisco Bay area (Fig. 3a). Figure 3b shows that within the Bay area the seismic deformation varies locally (see also Table 1). In almost all local regions the strain patterns are independent of earthquake size. A comparison with the geodetic strain pattern in these small areas is difficult because the geodetic data lacks sufficient resolution²⁵. The seismic strain patterns, however, are associated with local tectonics and can partly be predicted from the geometry of the major strike-slip faults. In regions where they are oriented in the plate motion direction we expect shear, as observed for example in the Calaveras area. In regions where the trend of the faults is more westerly or more easterly we expect net contraction or net extension. This is observed along the San Andreas fault. In the San Francisco Peninsula, the Loma Prieta and the Hollister regions (although less prominent there), where the fault strikes more westerly than the plate motion, the seismic strain is contractional. Around the Golden Gate, where the fault strikes more easterly, the seismic strain is extensional. These strain patterns are compatible with the uplift of the southern Santa Cruz Mountains and the subsidence of the Golden Gate area, respectively²⁵.

Earthquake focal mechanisms are commonly interpreted with respect to the state of stress in the Earth's crust^{26–28}. Here we have been concerned with the state of strain. In homogeneous, isotropic material the elastic strain results in a stress state with identical principal directions. In the Earth's brittle crust, however, they should be different because the strain localizes on fractures or faults on a range of scales. In determining strain by summing over many events and over a large period of time we average over strain heterogeneities and reproduce the applied deformation. Stress inversion assumes that the earthquakes are driven by a single stress tensor invariant in space and time, and if the same strain and stress directions are obtained from focal mechanism data this is fortuitous. In the Loma Prieta region, for example, the strain directions differ from stress directions previously reported^{29–31}, possibly because the hypothesis of a spatially and temporally uniform stress tensor is not met. □

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A snake with legs from the marine Cretaceous of the Middle East

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Although snakes are descended from limbed squamates ('lizards'), all known snakes lack well developed legs and their nearest lizard relatives have yet to be identified^{1–4}. Here we provide compelling evidence that the Cretaceous squamate *Pachyrhachis problematicus*, previously interpreted as a varanoid lizard^{5–7}, is actually a primitive snake with a well developed pelvis and hindlimbs. *Pachyrhachis* is the sister-taxon of all other snakes. The skull exhibits most derived features of modern snakes, and the body is slender and elongated. But unlike other snakes, *Pachyrhachis* retains a well developed sacrum, pelvis and hindlimb (femur, tibia, fibula, tarsals). *Pachyrhachis* was marine, and provides additional support for mosasauroid-snake affinities.

Both specimens of *Pachyrhachis problematicus* were found in limestone quarries at Ein Jabrud (Bed-Meir Formation, lowermost Cenomanian, mid-Cretaceous⁸), 20 km north of Jerusalem, Israel. The first was named *Pachyrhachis problematicus* (HUI-PAL 3569)^{5,6} and the second *Ophiomorphus colberti* (HUI-PAL 3775)⁷. *Ophiomorphus* was pre-occupied, and thus changed to *Estesius*⁹. Some snake-like features were noted in both but it was concluded that they were long-bodied varanoid lizards and probably not closely related to snakes^{6,7}. Our re-study found no significant differences between the two specimens (Figs 1 and 2). The posterior region of *Pachyrhachis* is not preserved, resulting in the reported absence of the hindlimb⁷ (Fig. 3). *Pachyrhachis* is slightly larger and

has slightly more pronounced pachyostosis. Apart from this, the two animals are indistinguishable and can be united on the basis of several unique derived features, for example, anteroposteriorly expanded quadrate, large rectangular coronoid process, anteriorly located intramandibular joint, and pachyostotic mid-dorsal vertebrae and ribs. The small difference in pachyostosis is likely to be ontogenetic, and *Estesius* (*Ophiomorphus*) *colberti* is here made a junior synonym of *Pachyrhachis problematicus*.

Pachyrhachis has a small, narrow, lightly built skull (Figs 1 and 2) showing most derived features of modern snakes. Most elements typical of squamates³ are present. However, the lacrimal is absent, and the postorbital and postfrontal are fused. The prefrontal has a flange that extends posteriorly to contact the parietal. The frontal is long and narrow, and bears a descending flange that forms the anterior margin of the optic foramen. The ventral edge of the descensus parietalis is exposed in the holotype and meets the parabasisphenoid (Fig. 1b). The braincase therefore appears to be fully enclosed in bone, although the descensus parietalis is not exposed in all places. The parietal table is reduced to a narrow sagittal crest, indicating that the external jaw adductors originated along the entire dorsal surface of the parietal. The supratemporal is long, narrow, and superficial to the parietal (Figs 1 and 2). The suspensorial ramus of the parietal is absent, and the quadrate is suspended entirely by the supratemporal (Fig. 1a). The quadrate is

vertically oriented and has a large flat external surface that lacks a tympanic recess.

The skull is highly kinetic. The anterior end of the maxilla is smoothly rounded and is not sutured to the premaxilla, suggesting a mobile contact (Fig. 1c). The upper temporal arch is absent, and the quadrate is streptostylic. The tips of the dentaries are rounded, indicating a mobile mandibular symphysis (Fig. 1c). A highly mobile, vertical splenial-angular ('intramandibular') joint is present. The loose articulation of the pterygoids with the palatines, and the absence of restrictive basipterygoid processes, suggests palatal kinesis. Long, recurved teeth are present on the palatine, pterygoid, dentary, maxilla, and presumably the premaxilla. These teeth are weakly fluted basally, have labial and lingual carinae, and are ankylosed to the rims of shallow sockets (Fig. 1b).

The body is very long and slender. There are 18 cervical-like vertebrae, bearing blade-like hypapophyses, that form a long slender neck-like region. The trunk has approximately 128 dorsal vertebrae, giving a total of 146 presacals. The vertebrae of *Pachyrhachis* are all articulated: zygosphenes-zygantra articulations are visible but their morphology cannot be ascertained. Mid-dorsal vertebrae and ribs are pachyostotic. The tail is not preserved in either specimen.

The shoulder girdle and forelimb are absent. However, the pelvis and hindlimb are present, preserved only in the second specimen. The ilium, ischium and pubis are separate elements loosely attached

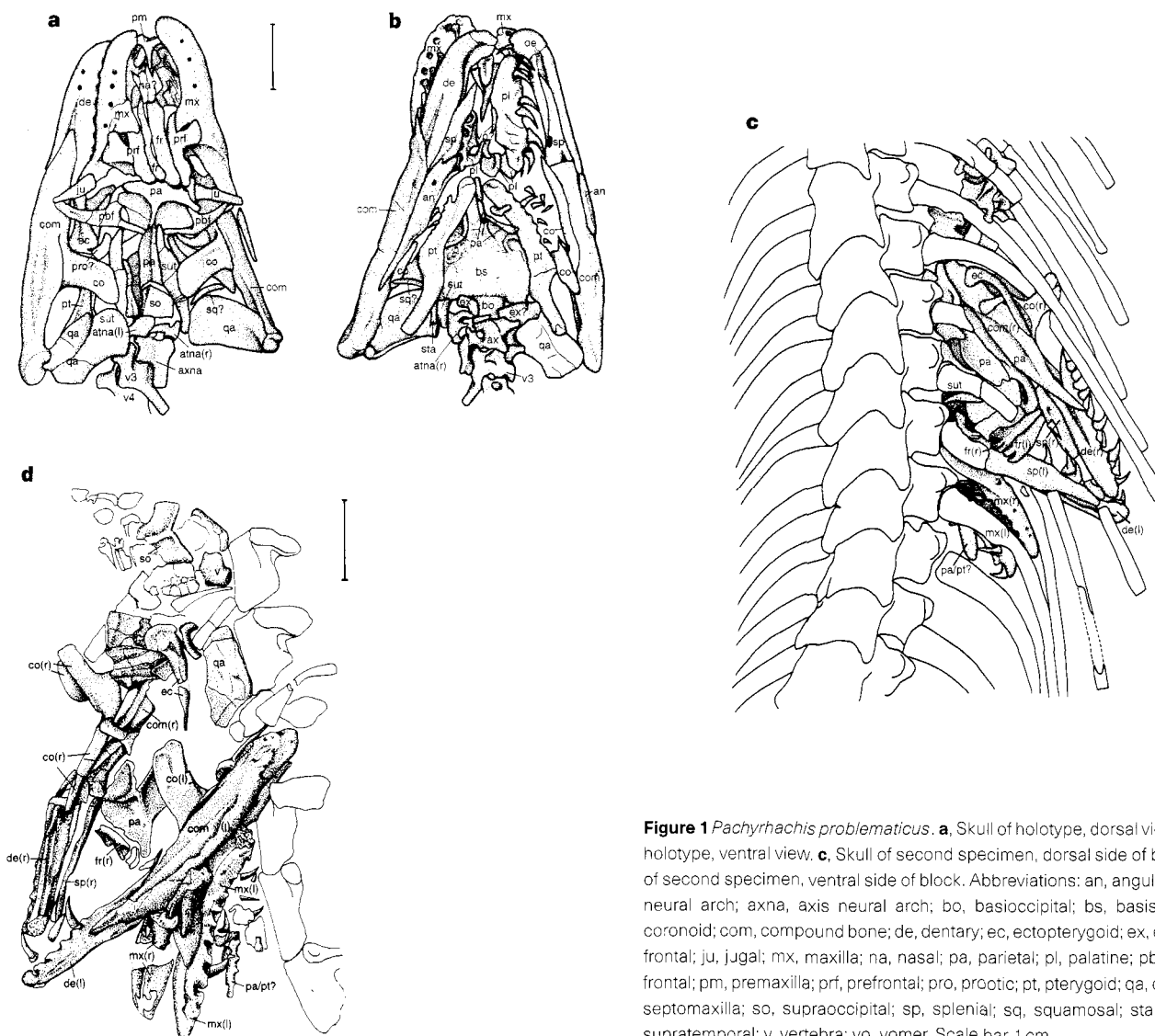


Figure 1 *Pachyrhachis problematicus*. **a**, Skull of holotype, dorsal view. **b**, Skull of holotype, ventral view. **c**, Skull of second specimen, dorsal side of block. **d**, Skull of second specimen, ventral side of block. Abbreviations: an, angular; atna, atlas neural arch; axna, axis neural arch; bo, basioccipital; bs, basisphenoid; co, coronoid; com, compound bone; de, dentary; ec, ectopterygoid; ex, exoccipital; fr, frontal; ju, jugal; mx, maxilla; na, nasal; pa, parietal; pl, palatine; pbf, postorbitofrontal; pm, premaxilla; prf, prefrontal; pro, prootic; pt, pterygoid; qa, quadrate; sm, septomaxilla; so, supraoccipital; sp, splenial; sq, squamosal; sta, stapes; sut, supratemporal; v, vertebra; vo, vomer. Scale bar, 1 cm.

to each other. The ilium articulates with the distal end of at least one sacral rib, possibly two. The hindlimb is represented by a femur, tibia, fibula, astragalus and calcaneum (Fig. 3c, d). Unlike most squamates, but as in mosasauroids¹⁰, the astragalus and calcaneum are separate.

Pachyrhachis exhibits all the synapomorphies of squamates for which it can be coded^{3,11} (Fig. 4). The major question concerns the position of *Pachyrhachis* within Squamata and the supporting characters. We addressed this by a cladistic analysis of *Pachyrhachis*, scolecophidian snakes, alethinophidian snakes, and 22 other major groups of fossil and living squamates (Fig. 4). *Pachyrhachis* and modern snakes (scolecophidians and alethinophidians) form a robust ophidian clade to the exclusion of all other squamates. Many of the derived characters uniting *Pachyrhachis* and modern snakes (Fig. 4) were previously thought to be unique to modern snakes^{1-3,12,13}. Within ophidians, *Pachyrhachis* retains several lizard-like features lost in all modern snakes (Fig. 4)^{2,3,12-14}, indicating that it is the most primitive snake and the sister group to modern snakes.

Snakes are widely thought to have evolved from small fossorial lizard ancestors^{2,4,12,14-16}, though an aquatic origin has been proposed^{17,18}. Extant, highly fossorial taxa such as scolecophidians, *Anomochilus*, uropeltids, cylindrophids and aniliids are considered to be the most primitive snakes, and other snakes are postulated to

be secondarily surface-living^{2,4,12,14-16}. *Pachyrhachis*, however, is marine. Both specimens, found in marine limestones⁸, are fully articulated, indicating *in situ* preservation. The pachyostotic trunk vertebrae and ribs also indicate an aquatic existence. The separate astragalus and calcaneum (Fig. 3), suggesting reduced limb ossification, are observed only in marine squamates¹⁰ and further support the conclusion that this snake was marine. The recognition that the most primitive snake is marine does not support the fossorial hypothesis, and suggests that an aquatic ancestry for snakes merits serious reconsideration.

Pachyrhachis sheds new light on the long-standing problem of the nearest lizard relatives ('ancestors') of snakes, by providing important new information on primitive ophidian character states. A recent study has found equivocal evidence uniting snakes with other limbless squamates such as amphisbaenians and dibamids¹⁹. However, other analyses of squamate phylogeny^{13,20,21} have revived an old idea¹⁷ by finding derived characters linking snakes and mosasauroids (Cretaceous marine lizards such as aigialosaurs, mosasaurs and coniasaurs²²). While these traits are present in all (or at least most) snakes, many are particularly well developed in *Pachyrhachis*, here demonstrated to be the most primitive snake: quadrate suspended entirely by supratemporal, reduced basiptyergoid processes, continuous supraoccipital-parietal suture, long recurved pterygoid teeth, surangular not exposed on medial surface of lower jaw,

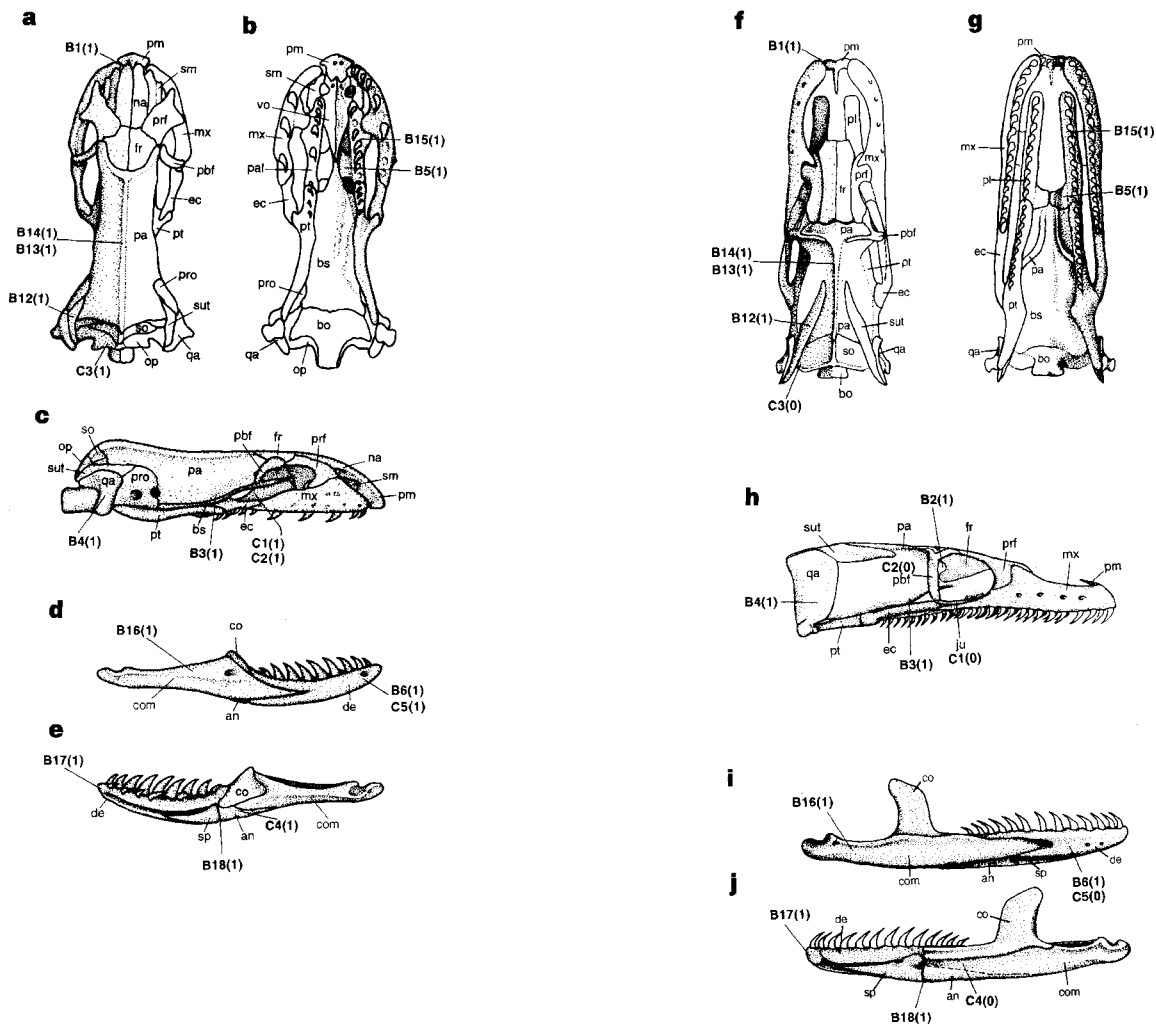


Figure 2 Comparative views of a primitive snake, *Cylindrophis* (Australian Museum R131356), and *Pachyrhachis*. Skull of *Cylindrophis* in (a) dorsal, (b) ventral, and (c) lateral view. Lower jaw of *Cylindrophis* in (d) lateral and (e) medial

view. Skull of *Pachyrhachis* in (f) dorsal, (g) ventral, and (h) lateral view. Lower jaw of *Pachyrhachis* in (i) lateral and (j) medial view. Numbers refer to derived characters discussed in Methods. Abbreviations as in Fig. 1.

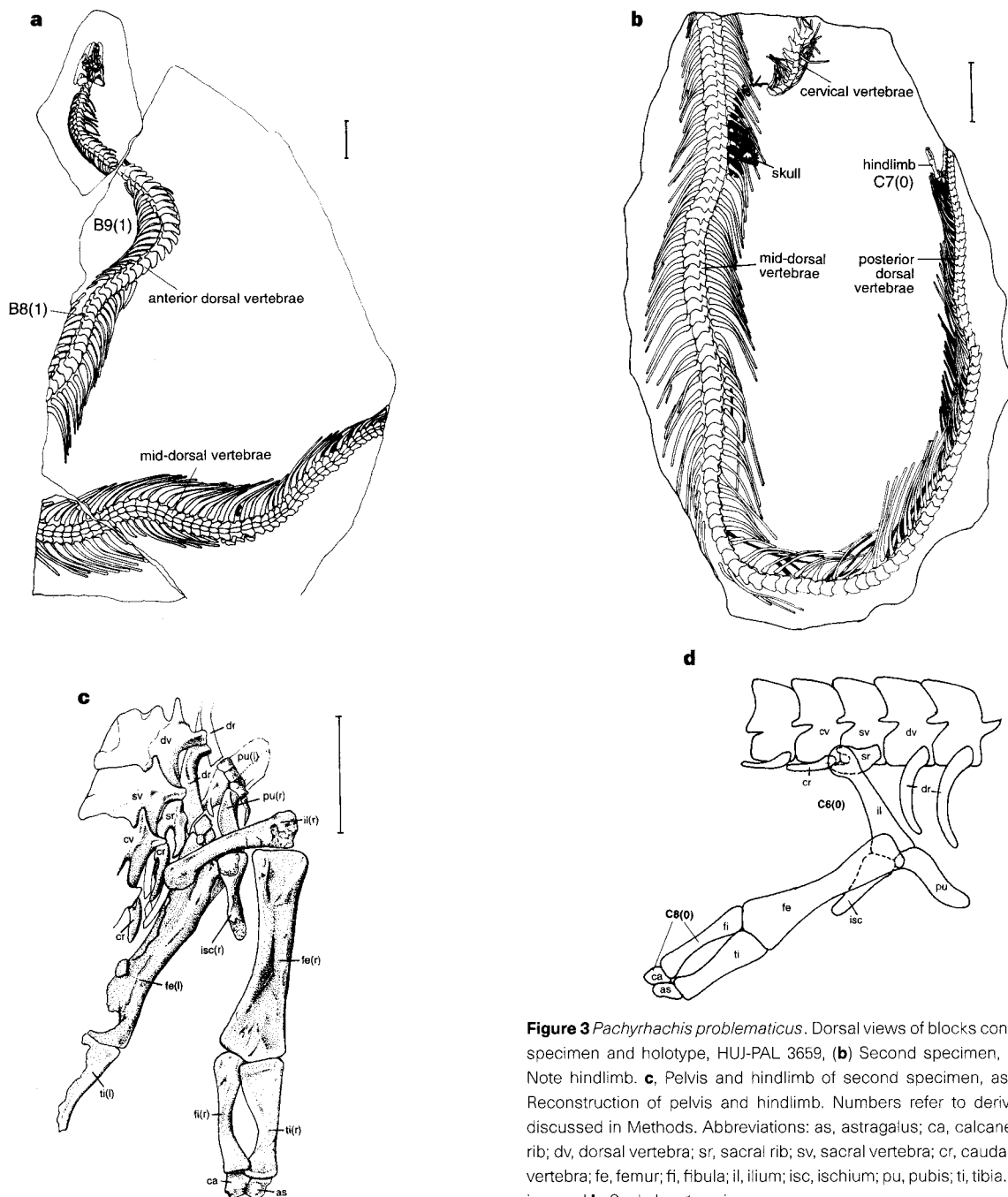
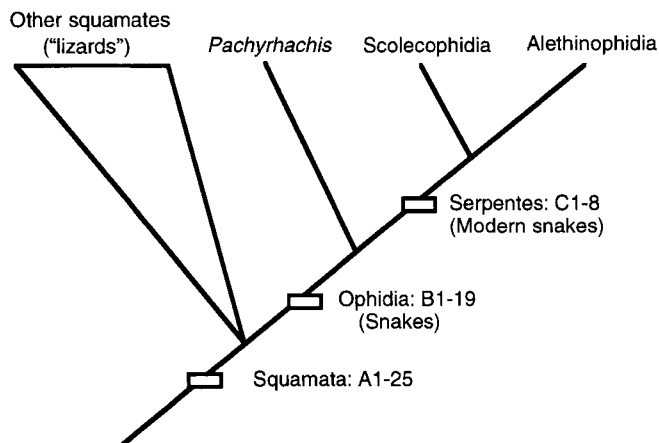


Figure 3 *Pachyrhachis problematicus*. Dorsal views of blocks containing (a) First specimen and holotype, HUI-PAL 3659, (b) Second specimen, HUI-PAL 3775. Note hindlimb. c, Pelvis and hindlimb of second specimen, as preserved. d, Reconstruction of pelvis and hindlimb. Numbers refer to derived characters discussed in Methods. Abbreviations: as, astragalus; ca, calcaneum; dr, dorsal rib; dv, dorsal vertebra; sr, sacral rib; sv, sacral vertebra; cr, caudal rib; cv, caudal vertebra; fe, femur; fi, fibula; il, ilium; isc, ischium; pu, pubis; ti, tibia. Scale bar 5 cm in a and b; Scale bar, 1 cm in c.

Figure 4 The phylogenetic affinities of *Pachyrhachis*. The interrelationships of 'lizards' (almost certainly paraphyletic²⁻³), dibamids, and amphisbaenians are beyond the scope of this paper, and they have been depicted as an unresolved polytomy within Squamata ('other squamates'). The numbered traits diagnosing the indicated clades are described under Methods, and selected traits diagnosing Ophidia and Serpentes are illustrated in Figs 1-3.



mobile mandibular symphysis, straight and vertical splenial–angular joint, pelvic elements not suturally united. Furthermore, in contrast to all other squamates, in mosasauroids^{10,22} and *Pachyrhachis* the astragalus and calcaneum are separate (this character is not applicable in other snakes, which have lost the tarsals). The morphology of *Pachyrhachis* therefore provides surprising and compelling new evidence for the hypothesis that, among lizards, the marine mosasauroids are the nearest relatives of snakes^{13,17,20,21}. □

Methods

Listed below are derived characters (synapomorphies) diagnosing clades within Squamata shown in Fig. 4, supporting the interpretation of *Pachyrhachis* as the sister group to all other snakes. This conclusion was supported by a cladistic analysis of all squamates, including dibamids and amphisbaenians (see Supplementary Information). For each character, the derived condition diagnosing the relevant clade is listed first, the primitive condition characterizing taxa outside the clade is listed afterwards in parentheses.

SQUAMATA. (See refs 3, 11 for more complete descriptions.)

A1 premaxillae fused (separate); **A2** frontoparietal suture a straight transverse line (suture W- or U-shaped); **A3** parietals fused (separate); **A4** parietal table short, braincase exposed dorsally behind table (long, braincase not exposed); **A5** anteroventral border of orbit formed by jugal (border formed by maxilla)—this trait is reversed in many squamates³; **A6** quadratojugal absent (present); **A7** posterior process of jugal absent (present); **A8** ventral ramus of squamosal absent (present) (A6–A8 all occur in kuehneosaurs and thus might be primitive for lepidosaurs³); **A9** quadrate lacks anteromedial or pterygoid lappet (lappet present); **A10** pterygoids completely separated in midline (pterygoids meet); **A11** pterygoid broadly enters suborbital fenestra (pterygoid largely or completely excluded); **A12** palatine with choanal groove (choanal groove absent). The medial process of the palatine in *Pachyrhachis* and other snakes is concave (grooved) ventrally; **A13** stapes very slender (stapes robust); **A14** angular short, not extending posteriorly to the level of mandibular condyle (angular long); **A15** coronoid process large and formed entirely by coronoid bone (coronoid process, if present, formed partly by surangular); **A16** proatlas absent (present); **A17** vertebrae procoelous (not procoelous); **A18** cervical and posterior trunk ribs single-headed (double-headed); **A19** cervical intercentra modified into prominent, blade-like hypapophyses (hypapophyses absent); **A20** eight or more cervical vertebrae (seven or fewer cervicals). *Pachyrhachis* lacks a shoulder girdle and thus an obvious cervical region. However, the first 18 vertebrae are all 'cervical-like', being small and bearing prominent hypapophyses; **A21** posterior trunk intercentra absent (present); **A22** pelvic thyroid fenestra enlarged, pubic symphysis very short (fenestra small); **A23** absence of tibial ridge and astragalar groove in tibio-astragalar joint (ridge and groove present); **A24** fibulo-astragalocalcaneal joint involves most of distal end of fibula (does not involve most of distal end of fibula); **A25** gastralia absent (present).

OPHIDIA (All snakes: *Pachyrhachis*, scolecophidians, and alethinophidians.)

Unequivocal characters: **B1** premaxilla–maxilla articulation non-sutural and mobile (contact rigid and sutural); **B2** descending process of frontal forming part or all of margin of optic (II) foramen (foramen not enclosed in bone); **B3** descending process of parietal meeting parabasisphenoid (parietal does not contact parabasisphenoid); **B4** tympanic recess absent (recess present); **B5** palatine with distinct rectangular medial process which meets its partner in the midline (process absent); **B6** dentary with two or fewer mental foramina (three or more foramina); **B7** marginal teeth ankylosed to the rims of discrete sockets (sockets absent in all lizards, including primitive mosasauroids. In derived mosasauroids sockets are present, but the teeth are deeply inserted into the sockets rather than ankylosed to the rims^{22,23}); **B8** body long, more than 140 preloacal vertebrae (body short, fewer than 140 preloacal vertebrae); **B9** shoulder girdle and forelimb completely absent (girdle present, even in limb-reduced lizards); **B10** epiphyses absent (present). **Equivocal characters.** In addition, *Pachyrhachis* and modern snakes share the following synapomorphies which, however, are less compelling as they each occur in a few other squamates. **B11** lacrimal absent (lacrimal present); **B12** supratemporal superficial to parietal (supratemporal partly covered by suspensorial ramus of parietal)—this does not occur in any other squamate, but is equivocal because it is not applicable in scolecophidians and some alethinophidians, which have

lost the supratemporal; **B13** external jaw adductors insert on dorsal surface of the parietal (jaw adductors insert mainly on ventral surface, and lateral margins, of parietal); **B14** parietal table reduced to sagittal crest (parietal table wide); **B15** palatine teeth long and recurved (palatine teeth small denticles)—not applicable in taxa without palatine teeth; **B16** surangular, prearticular and articular fused into a single compound postdentary bone (surangular, prearticular, and articular separate); **B17** highly mobile mandibular symphysis, dentaries with rounded anterior tips (rigid mandibular symphysis, dentaries with large symphyseal surfaces); **B18** 'intramandibular joint'—angular and splenial with vertical articular facets and no overlap (overlap extensive, medial and lateral flanges variably present on both elements); **B19** well-developed zygosphenes and zyganchtra (zygosphenes and zyganchtra absent).

SERPENTES (Crown-clade snakes: scolecophidians and alethinophidians.)

C1 jugal lost (jugal present); **C2** posterior orbital margin incomplete (margin complete). Also occurs convergently in certain lizards (for example, *Varanus*); **C3** exoccipitals (or fused exoccipital–opisthotics) meet above foramen magnum (exoccipitals not meeting)—although the dorsal portions of the exoccipitals are not visible in *Pachyrhachis*, the finished posterior margin of the supraoccipital indicates that it possessed the primitive character state; **C4** angular–coronoid contact present on medial surface of lower jaw (contact absent); **C5** single mental foramen on dentary (*Pachyrhachis* has two foramina, other squamates have three or more); **C6** pelvis—when present—lies within ribcage, sacral contact absent (pelvis external to ribcage, sacral contacts usually present); **C7** femur vestigial or lost (in *Pachyrhachis*, the femur, though small, is larger than in any other snake); **C8** tibia, fibula, astragalus and calcaneum lost (all retained in *Pachyrhachis*).

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Supplementary Information is available on Nature's World-Wide Web site (<http://www.nature.com>) or as paper copy from Mary Sheehan at the London editorial office of Nature.

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Insect parasitoid species respond to forest structure at different spatial scales

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There is now a solid body of theoretical work^{1–4} demonstrating that the spatial structure of the habitat combined with animal movement strongly influence host–parasitoid dynamics. The spatial pattern over which parasitoid search takes place can be affected by the distribution of the hosts⁵, by the spatial arrangement of the host's habitat⁶ and by the spatial scale at which the parasitoid perceives variation in host abundance^{7,8}. Empirical work, however, has been largely restricted to small-scale field studies of less than one hectare^{6,9} with very few larger^{10,11}. Here we report initial results of a many-year, large-scale study that is among the first to examine the interaction between a population-level process (parasitism) and anthropogenic forest fragmentation at large and at multiple spatial scales. We demonstrate that parasitism by four species of parasitoids attacking the forest tent caterpillar, *Malacosoma disstria*, is significantly reduced or enhanced depending on the proportion of forested to unforested land. Each of the parasitoid species responds to this mosaic at four different spatial scales that correspond to their relative body sizes. Our data give empirical support to the argument that changes in landscape structure can alter the normal functioning of ecological processes such as parasitism, with large-scale population consequences^{3,4}.

We determined the effect of forest structure (mosaic of cleared and forested land) on the rates of parasitism caused by four species of parasitic fly attacking an outbreak population of the forest tent caterpillar (*Malacosoma disstria*) in aspen forests near Edmonton, Alberta, Canada. Studies were done on a large-scale grid 420 km² in area (127 sample points) and a small-scale grid of 0.32 km² area (109 sample points) nested within the larger grid. At each sample point we estimated host population density, rates of parasitism caused by each species, and forest structure. Forest structure around each point was estimated at seven scales, from 53 m around each point up to 3,400 m.

Univoltine fly parasitoids dominate the parasitoid community that attacks forest tent caterpillar^{12–14}. The four species we studied ranged in size from 34 mg for *Carcelia malacosomae* (Tachinidae) which attack host larvae directly, 41 mg for *Patelloa pachypyga* and 68 mg for *Leschenaultia exul* (Tachinidae) which lay eggs on foliage that are subsequently ingested by feeding host caterpillars, and 58 mg for *Arachnidomyia* (= *Sarcophaga*) *aldrichi* (Sarcophagidae) which larviposits on host cocoons. The three largest of these species are considered important in suppressing tent caterpillar outbreaks^{12–14}. Because larvae of all of these flies develop in the late larval and pupal stage of the host there is some competition among them. *A. aldrichi* attacks host pupae and is the last of the four to attack during the host's life cycle. Therefore, it competes with *C. malacosomae* and especially with *P. pachypyga* which remain within the host into the host's pupal stage^{12–14}. Larvae of *L. exul* exit before host pupation and are therefore affected little by competition with *A. aldrichi*¹⁴.

Parasitism by all species varied markedly across the study area (Fig. 1) in response to both forest structure and the abundance of hosts (Table 1). Parasitism by the three largest fly species was

greatest on hosts collected in contiguous forests, and was lower in those from fragmented forests (Table 1; positive coefficients reflect greater parasitism in contiguous forest). However, the spatial scale at which forest structure had its greatest effect differs among the parasite species. Parasitism by the largest species, *L. exul*, was most strongly correlated with forest structure measured at a scale of 850 m around each site. Parasitism by medium-sized flies *A. aldrichi* and *P. pachypyga* was most strongly related to forest structure within 425 m and 212 m, respectively. They caused less parasitism in the areas of greatest forest fragmentation (Fig. 1) and along large forest edges (Fig. 2). In contrast, the smallest fly, *C. malacosomae*, caused higher rates of parasitism in the fragmented forests (Fig. 1) and at forest edges (Fig. 2), and was affected by forest structure at the finest spatial scale (53 m). Parameter estimates for the effects of forest structure were not significantly different when estimated from data

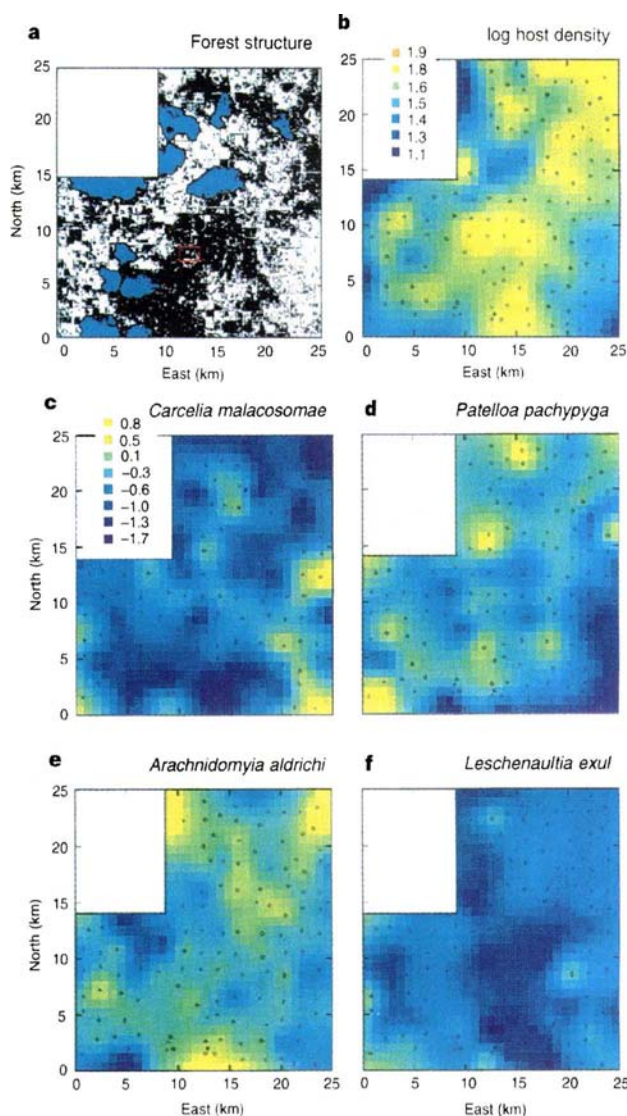


Figure 1 **a**, Forest structure, **b**, log host density in 1995 (N_t) and **c–f**, parasitism by four fly parasitoid species attacking forest tent caterpillar at Ministick Hills, Alberta, Canada. Maps of parasitism are distance-weighted least-squares surface plots²¹ for each of four parasitoid species attacking tent caterpillar across the coarse-resolution grid: **c**, *C. malacosomae*; **d**, *P. pachypyga*; **e**, *A. aldrichi*; and **f**, *L. exul*. Colours indicate log host density (**b**) and log odds-ratios of number parasitized by each species against number not parasitized (**c–f**). Dots are the 127 population sample sites. The red rectangle (in **a**) indicates the location of the fine-resolution grid (Fig. 2).

from the coarse-resolution and fine-resolution grids. The similarity of parameter estimates from the grids with different resolution suggests that there are no additional effects of landscape across the large-scale grid beyond those that can be estimated at a given sample point.

Rates of parasitism across the fine-resolution grid were spatially autocorrelated. Again, the distance to which they were autocorrelated differed among the four species. Parasitism by the two smallest species, *C. malacosomae* and *P. pachypyga*, were autocorrelated to

distances of only 44 and 53 m, respectively, reflecting fine-grained variation in parasitism. Parasitism by the two larger fly species, *A. aldrichi* and *L. exul*, was autocorrelated to distances of 421 and 420 m, respectively, reflecting coarser-grained variation in parasitism. These patterns suggest greater movement by the larger fly species, reflected in the larger spatial scale at which they respond to forest structure.

In general, parasitism was higher in areas of high host density, a pattern most evident for *P. pachypyga* and *A. aldrichi*. Interestingly,

Table 1 Coefficients for data from the coarse-resolution grid

Parasitoid species	Pupal mass (mg)	53 m	106 m	212 m	425 m	850 m	1,700 m	3,400 m	N_{t-1}	N_t
<i>Carcelia malacosomae</i>	34	-0.77						0.10	-0.17	-0.26
<i>Patelloa pachypyga</i>	41		2.0					0.17		0.80
<i>Arachnidomyia aldrichi</i>	58			2.23					-0.12	0.52
<i>Leschenaultia exul</i>	68				1.85				-0.24	0.28

Coefficients from logistic regression models estimating the effects of forest structure (Fig. 1a) and host density (Fig. 1b) on the odds of being parasitized by each of four parasitoid species across the large, coarse-resolution grid. We show only the coefficients for the effects of forest structure at the spatial scale at which its effect was strongest, and for the effect of host density. Full models are contained in supplementary information.

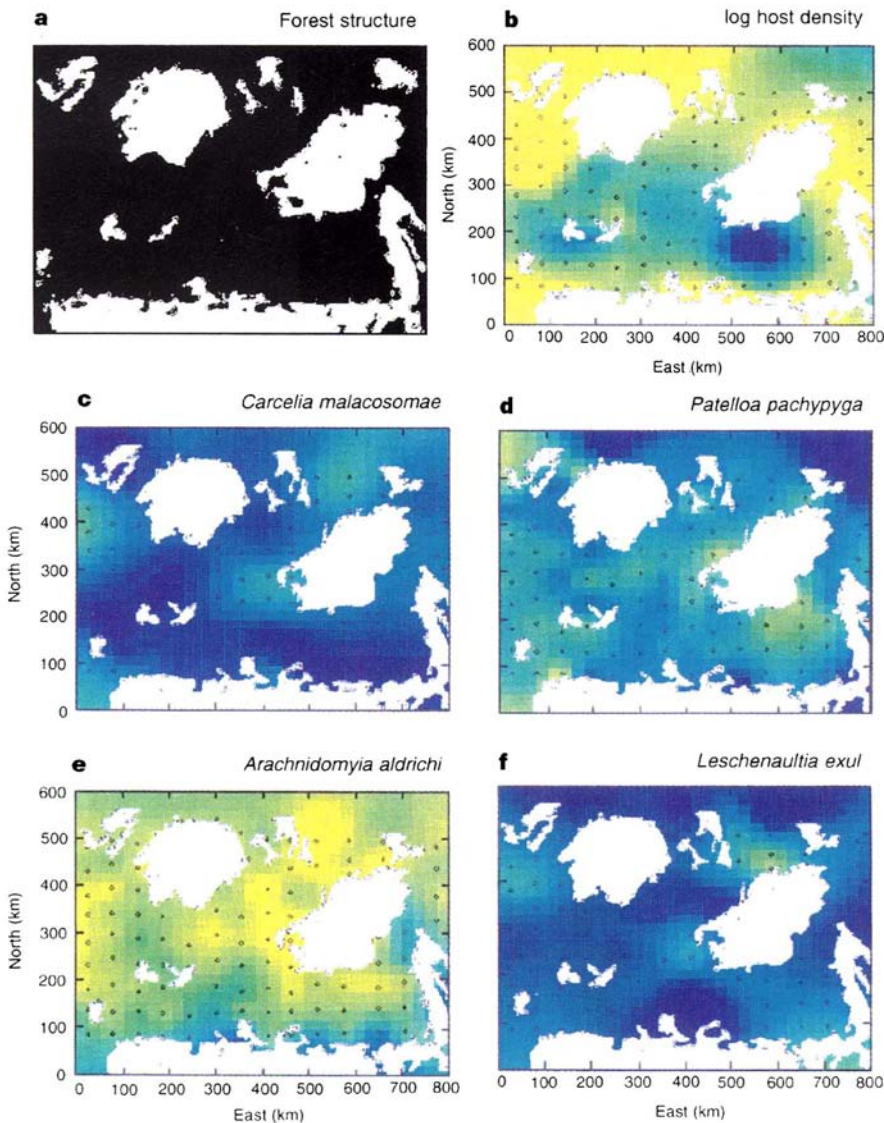


Figure 2 a, Forest structure, b, log host density in 1995 (N_t), and c-f, parasitism across the 0.32, m² fine-resolution grid. Parasitism is estimated for c, *Carcelia malacosomae*; d, *Patelloa pachypyga*; e, *Arachnidomyia aldrichi*; and f, *Leschenaultia exul*. Methods and symbols as in Fig. 1).

the smallest parasitoid species again showed a pattern opposite to that of the other three species, causing higher rates of parasitism in areas of low host density (Table 1). For the two smallest parasitoid species (*C. malacosomae* and *P. pachypyga*), there was a significant interaction between host density and forest structure on the rates of parasitism they cause. *C. malacosomae* responded positively to host density in partially cleared forests (at 53 m scale) but did not respond to host abundance in continuous forests. These patterns suggest that forest acts as a barrier to movement (aggregation) by *C. malacosomae*, or that their numbers increase little in continuous forest. *Patelloa pachypyga*, in contrast, responded strongly and positively to host abundance in continuous forests, and only weakly so in partially cleared forests, suggesting that clearings may inhibit movement by *P. pachypyga*. Theoretically, such limits on parasitoid redistribution undermine any stabilizing effect of parasitoid aggregation¹⁵. There were no such interactions for the two largest fly species, suggesting that any response to host abundance was consistent regardless of habitat structure.

Although our spatial analyses clearly show that the landscape patterning of forests influences the percentage of host attacked by parasites, the real question is whether this effect translates into altered host–parasitoid dynamics. Data from other studies suggest in fact that the effects of forest structure we document in this paper do indeed alter dynamics in a profound way. In particular, the three parasitoid species that are less effective in fragmented forests are those that normally dominate in declining populations of tent caterpillar in several parts of North America^{12–14}. Outbreaks of tent caterpillar last longer in fragmented than in continuous forest¹⁶. On the basis of our analysis, a reduction in forest cover from continuous to 50 per cent cover reduces the odds of parasitism by the three dominant parasitoid species to about half of that in intact forests. These species would be predicted to be most efficacious in only those areas with relatively large blocks of contiguous forests,

212 to 850 metres square in size (Fig. 3). The one parasitoid species that benefits from partial clearing of forests, *C. malacosomae* (Table 1), plays a minor role during tent caterpillar outbreak and collapse^{12,13}. Forest fragmentation, therefore, may exacerbate outbreaks of tent caterpillar by decoupling it from its natural enemies.

We suggest that the mechanism for altered parasitism is through the effects of habitat structure on movement. We have shown elsewhere¹⁷ that the larger species (*A. aldrichi*) can ‘colonize’ isolated forest stands up to 400 m from contiguous forest compared to only 125 m for the smaller species, *P. pachypyga*. Our analysis here also suggests that the ability of parasitoids to aggregate in response to host density is affected by landscape. It may be argued that forest fragmentation alters microclimate along forest edges, which different species may either favour or avoid¹⁸. However, we have controlled the effect of distance to edge at our large-grid sites by placing each sampling point 20 m into the forest; and we have removed the effect of distance to edge in the analysis of the fine-grid data. Therefore, although there may be an effect of edge, the fragmentation effect detected here is not directly related to the edge. Differences in spatial patterns seen among the fly species may reflect a strong spatial pattern for one species, for example *A. aldrichi*, combined with its ability to out-compete the other species. However, such an effect on another species would be expected to result in a response to forest structure in the opposite direction (higher parasitism in the fragmented stands where *A. aldrichi* does poorly) but at the same spatial scale (425 m). The most likely competitors with *A. aldrichi* either show the same (positive) direction of their response to forest structure (*P. pachypyga*) or respond to forest structure at a much finer scale (*C. malacosomae*).

Changes in landscape structure such as forest fragmentation have been predicted to affect animals differentially depending on their size and the spatial grain of the habitat mosaic¹⁹. Populations of animals that evolved a specific ‘ambit’ within, for example, a

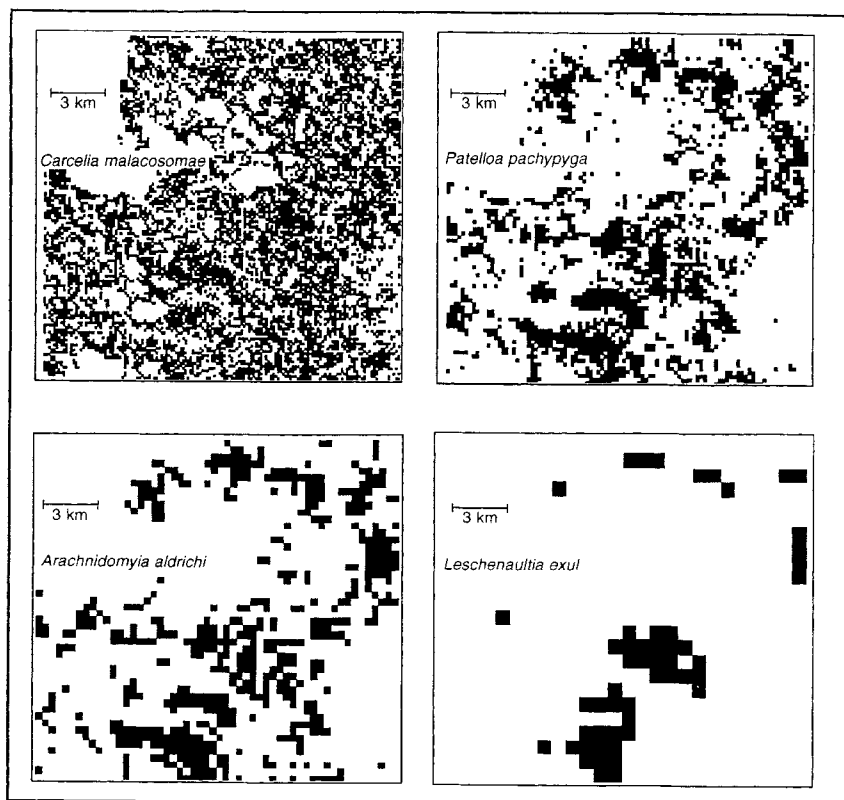


Figure 3 The extent of forested habitat in which each parasitoid species would be most effective, disregarding effects of host density. Patterns are based on the effects of forest structure on parasitism (positive or negative) and the spatial scale

at which each species responds to forest structure most strongly (from Table 1). Forests where the efficacy of each species is reduced from its maximum are deleted. Compare to Fig. 1a for the distribution of ail forest.

continuous forest system, may behave differently in areas of altered habitat structure. Successful predictions about the behaviour of complex systems and their response to disturbance may be possible using such simple relationships as those between animal size, spatial scale of search and grain of habitat structure. We reiterate the plea by others⁴ that ecological studies be conducted at sufficiently large and multiple spatial scales to successfully extract such generalizations. □

Methods

Estimates of parasitism and host abundance. At each sample point 50 to 100 late-instar caterpillars were collected to estimate parasitism by *C. malacosomae*, *P. pachygya* and *L. exul*, and 50 to 100 host cocoons were collected 10 days later to estimate rates of parasitism by *A. aldrichi*. The collection of late-instar hosts to estimate parasitism by *C. malacosomae* and *Patelloa pachygya*^{12–14} was made before the attack by *A. aldrichi*, which is known to out-compete the other parasites in the host pupa. Hosts from each collection were reared all together and the number of emerging parasitoids of each species were counted. Density was estimated using a time-restricted search; the time taken to collect 50 cocoons (to a maximum of 15 min) was recorded. If less than 15 min were needed to collect 50, we estimated the number which would have been collected in 15.

Estimates of forest structure. Forest structure was estimated at each of the sample points across the 420 m² grid. Landscape structure was estimated at each point from a classified aerial photo-mosaic (1:20,000 scale) scanned at a resolution of 5.3 m per pixel. Using SPANS Geographic Information System software (Intera Tydac Technologies, Nepean, Ontario, Canada), we calculated the amount of forest in a series of seven squares, geometrically increasing in spatial scale from 53 m on a side to 3,400 m on a side (Table 1) and centred on each sampling point. The amount of forest within each square was scaled from 0 (virtually no forest) to 1 (complete coverage of continuous forest); it is therefore an index of forest continuity.

Model fitting. For each parasitoid species, models included as potential explanatory variables: the log-transformed density of forest tent caterpillar in the previous year (N_{t-1} , 1994), the log-transformed density of forest tent caterpillar in the current year (N_t , 1995), and terms for the location of each sample point on a cartesian grid (east, north, coefficients not shown) and the square of these locations. Location terms were included to account for any unknown historical patterns of spread of the outbreak across the coarse-resolution grid. Model-building proceeded in a stepwise fashion by separately testing the importance of landscape structure measured at each of the seven spatial scales in each of seven separate models (each including density and location terms). We selected the model with the landscape term that caused the maximum reduction in overall deviance of the respective model, and for which the coefficient was most significantly different from zero²⁰. Once the best model was selected, additional terms were added to it to identify any additional effects of forest structure at scales smaller and larger than that which appeared to be most important. This strategy was used to identify the scale at which forest structure had its greatest effect, and then to examine whether additional terms contributed to the overall fit of the model. When testing for the effects of forest in the 3,400 m square surrounding a point, we used a subsample of points spaced by at least 4 km, thereby ensuring independence of forest structure estimates among points. Pupal mass was estimated from samples of 20 individuals of each fly species pooled from several sites. Full models are presented as supplementary information.

A second set of models (not shown) were fitted to data from the fine-resolution grid, but forest structure was only estimated within 53 m and 106 m around each sample point. Forest structure within 106 m of each point was again estimated using a subset of points to ensure independence of estimates. Distance of each sample point to the nearest forest edge was estimated and included in the model. Coefficients for the effect of forest structure (53 m and 106 m) on parasitism estimated from the fine-scale grid were not significantly different from those estimated from the coarse-resolution grid (53 and 106 m samples). All models were fit using S-PLUS software (MathSoft Inc., Seattle). Spatial autocorrelation of parasitism estimates for each species were based on the fit of a spherical model semivariogram with a minimum lag of 50 m, using GS+ geostatistics software (Gamma Design Software, Plainwall, Michigan).

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Arrest of spermatogenesis and defective breast development in mice lacking A-myb

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The *Myb* gene family currently consists of three members, named A-, B- and c-*myb*^{1,2}. These genes encode nuclear proteins that bind DNA in a sequence-specific manner and function as regulators of transcription. In adult male mice, A-*myb* is expressed predominantly in male germ cells^{2,3}. In female mice, A-*myb* is expressed in breast ductal epithelium, mainly during pregnancy-induced ductal branching and alveolar development. We report here that mice homozygous for a germline mutation in A-*myb* develop to

term but show defects in growth after birth and male infertility due to a block in spermatogenesis. Morphological examination of the testes of *A-myb*^{-/-} males revealed that the germ cells enter meiotic prophase and arrest at pachytene. In adult homozygous null *A-myb* female mice, the breast epithelial compartment showed underdevelopment of breast tissue following pregnancy and the female mice were unable to nurse their newborn pups. These results demonstrate that *A-myb* plays a critical role in spermatogenesis and mammary gland development.

To elucidate the functional and developmental role of *A-myb*, we used homologous recombination in embryonic stem (ES) cells to generate mice lacking a functional *A-myb* gene⁴. A 5.9-kilobase (kb) mouse genomic fragment was cloned from a murine 129/J library that was sequenced completely and found to contain exons III, IV and V, encoding the 5' end of the DNA binding domain of the A-Myb protein (Fig. 1a). To disrupt the DNA-binding domain of the *A-myb* gene, a targeting vector was designed in which the neomycin transferase (*NEO*) gene was inserted into exon IV at the *Clal* site (Fig. 1a). This vector was introduced into ES cells by electroporation, and double selection in G418 and gancyclovir produced 87 clones that were analysed by Southern blotting using a 700-base-pair (bp) *SspI* 5' *A-myb* probe (Fig. 1a). The appearance of an 8-kb band indicated a homologous recombination event in two of the clones analysed. The two clones bearing a disrupted *A-myb* gene were used to generate *A-myb*^{+/-} mice. Analysis of over 400 intercrosses between mice heterozygous for the disrupted *A-myb* allele revealed the presence of all three genotypes (+/+, +/- and -/-) (Fig. 1b) in the expected mendelian ratio of 1:2:1 (103:237:91). Northern blot analysis of RNA derived from the testes of these mice revealed that the *A-myb*^{+/+} and *A-myb*^{+/-} mice produced comparable levels of *A-myb* mRNA, but the *A-myb*^{-/-} mice failed to produce detectable *A-myb* mRNA (Fig. 1c). When western blots of testis homogenates were probed with a rabbit polyclonal antibody raised against the DNA-binding domain of the Myb protein, we

could readily detect a band corresponding to the 95K A-Myb protein in *A-myb*^{+/+} and *A-myb*^{+/-} homogenates, but there was no A-Myb protein in *A-myb*^{-/-} testis homogenates (Fig. 1d). These results demonstrate that disruption of the *A-myb* locus results in a complete loss of *A-myb* messenger RNA and protein in *A-myb*^{-/-} mice.

The first observable feature of the *A-myb*^{-/-} phenotype was the small size of the mice, apparent during the first few weeks of life. At birth, *A-myb*^{-/-} mice were indistinguishable from their littermates, but they lagged behind in their growth during the first few weeks after birth. In addition, the *A-myb*^{-/-} pups were small and wrinkled and had a hunched posture when compared to their littermates. As these *A-myb*^{-/-} mice matured (up to four months), these features became less pronounced and these mice eventually attained a body size comparable to that of the *A-myb*^{+/+} and *A-myb*^{+/-} mice (about 90% for the females and 70% for the males) (data not shown).

When *A-myb*^{-/-} male mice were mated with female mice, they copulated normally, as evidenced by the formation of vaginal plugs in their mates, but none of the female mice mated to *A-myb*^{-/-} males became pregnant. Sperm counts, using whole testis preparations⁵, showed that there was a complete absence of spermatozoa in *A-myb*^{-/-} testes, whereas *A-myb*^{+/+} mice contained $23.66 \pm 1.1 \times 10^6$ spermatozoa per testis and *A-myb*^{+/-} mice contained $22.8 \pm 1.26 \times 10^6$ spermatozoa per testis. An analysis of serum testosterone levels in *A-myb*^{+/+}, *A-myb*^{+/-} and *A-myb*^{-/-} mice revealed no significant difference between the three groups (data not shown). The size and weight of the testes from *A-myb*^{-/-} mice was ~25% of those of their littermates (Table 1), irrespective of age. Histopathological analysis of the testes of *A-myb*^{-/-} mice showed that there was a slight reduction in the size of the seminiferous tubules, although the number of tubules per testis appeared to be normal. Figure 2 shows sections of seminiferous tubules from wild-type and *A-myb*^{-/-} mice. The full complement

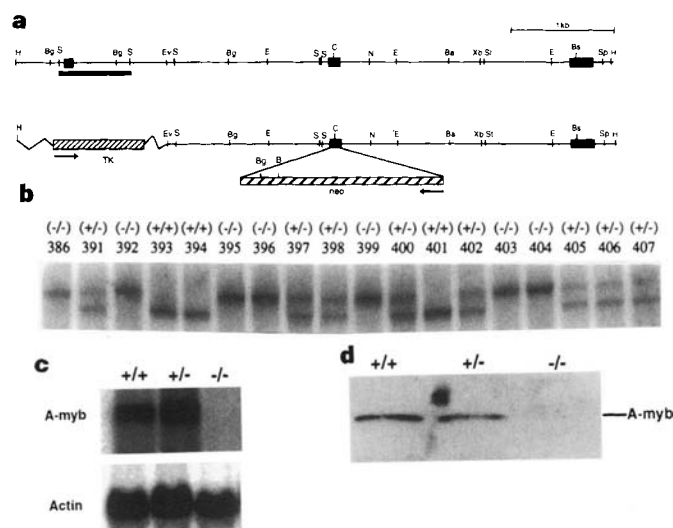


Figure 1 Targeted disruption of the mouse *A-myb* gene. **a**, Restriction map of the mouse *A-myb* genomic clone, and the structure of the targeting vector. Coding exons are depicted by black boxes. The genomic fragment used as a probe is shown as a black bar. B, *Bam*HI; Ba, *Ban*I; Bg, *Bgl*II; Bs, *Bst*XII; C, *Clal*; E, *Eco*RI; EV, *Eco*RV; H, *Hind*III; N, *Nco*I; S, *Ssp*I; sp, *Sph*I; St, *Stu*I; Xb, *Xba*I. **b**, Southern blot analysis of the DNA extracted from mouse tails. **c**, Northern blot analysis of total RNAs prepared from *A-myb*^{+/+}, *A-myb*^{+/-} and *A-myb*^{-/-} testes using *A-myb* and actin-specific probes². **d**, Western blot analysis of extracts prepared from *A-myb*^{+/+}, *A-myb*^{+/-} and *A-myb*^{-/-} testes for A-Myb expression.

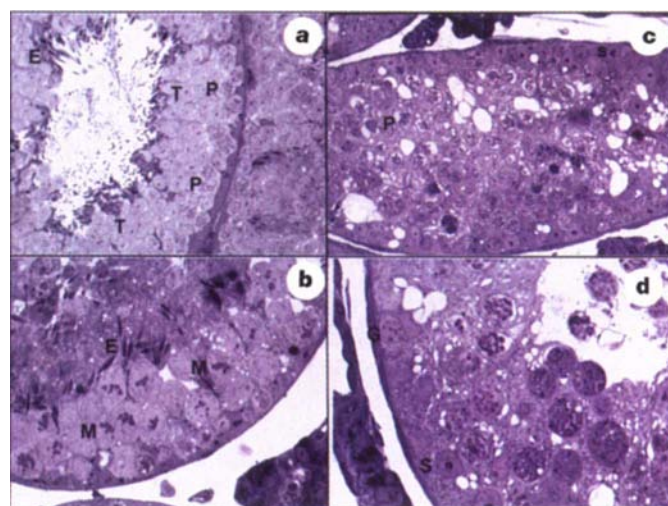


Figure 2 *A-myb*^{-/-} mice show defects in spermatogenesis. **a-d**, Sections of seminiferous tubules from either wild type (**a, b**) or *A-myb*^{-/-} mice (**c, d**) are shown. Primary spermatocytes in pachytene (P), round spermatids (T), primary spermatocytes in metaphase of the first meiotic division (M). Two tubules are shown in **a**: the one of the left is in stage IV-VII and the one of the right is in stage V. In **b**, the tubule is in stage XII. Note the absence of post-meiotic cells, such as spermatids or spermatozoa in **c** and **d**. Original magnification: **a** and **c**, $250 \times$; **b**, $500 \times$; **d**, $1,000 \times$. E, maturing haploid spermatids with elongated nuclei; G, primary spermatogonia; M, primary spermatocytes in metaphase of the first meiotic division; P, primary spermatocytes at pachytene; S, Sertoli cell nuclei; T, round spermatids.

of spermatogenic cells can be seen in the testes of the wild-type animals, including primary spermatocytes in pachytene (P) and round spermatids (T), as shown by the two tubules in Fig. 2a, the one on the left being in stage VI–VII and on the right being in stage V. Fig. 2b, a tubule in stage XII can be identified by the presence of primary spermatocytes in metaphase of the first meiotic division (M). Cross-sections of *A-myb*^{+/-} testes showed identical features (data not shown). In contrast, the morphology of the seminiferous tubules in *A-myb*^{-/-} mice was abnormal, with vacuolization of the Sertoli cell cytoplasm and degeneration among the population of primary spermatocytes (Fig. 2c, d). Spermatogonia and pre-leptotene spermatocytes were normal (G in Fig. 2d) and spermatogonial mitoses were commonly observed, whereas most pachytene primary spermatocytes showed different degrees of degeneration, from early nuclear changes (increased chromosomal density, for example) to advanced stages of cellular breakdown. Most notably, there was a complete absence of post-meiotic cells such as spermatids or spermatozoa, indicating that spermatogenesis in these mice came to an abrupt halt at the pachytene stage of meiosis. Analysis of cross-sections of these testes for the presence of apoptotic cells by TUNEL (for Tdt-mediated dUTP-biotin nick end labelling) assay⁶ showed that there were large numbers of apoptotic cells in *A-myb*^{-/-} testes; these cells were very rare in the *A-myb*^{+/+} and *A-myb*^{+/-} cross-sections (data not shown). Loss of *A-myb* did not seem to affect the formation of Leydig cell populations as they appeared to be normal in *A-myb*^{-/-} mice.

We next analysed testis RNA derived from *A-myb*^{+/+}, *A-myb*^{+/-} and *A-myb*^{-/-} mice for markers of testis development^{7,8}. Figure 3a shows that testis RNA preparations from *A-myb*^{-/-} mice lacked detectable CREM, *c-abl*, transition proteins 1 and 2 and protamine 1 and 2, although these were readily detectable in *A-myb*^{+/+} and *A-myb*^{+/-} mice. Because these genes are normally expressed during the post-meiotic stages of testis development^{7,8}, these results confirm the absence of spermatids and spermatozoa in *A-myb*^{-/-}

mice. It has been shown that post-meiotic cells express a truncated actin transcript of 1.5 kb, which seems to be produced by alternative splicing^{9,10}. Our analysis reveals that these alternatively spliced transcripts are absent in *A-myb*^{-/-} testes, whereas the normal transcript of 2.1 kb, produced in primary spermatogonia, is present in equal amounts in *A-myb*^{+/+}, *A-myb*^{+/-} and *A-myb*^{-/-} mice. Lack of transcription of alternatively spliced actin further indicates the absence of detectable spermatids or spermatozoa in *A-myb*^{-/-} mice. *c-mos*, which is expressed in spermatids as well as in Sertoli and Leydig cells⁸, was expressed at low levels in *A-myb*^{-/-} testis (Fig. 3b), presumably as a result of its presence in Sertoli and Leydig cells.

We next analysed the expression of genes that are transcribed before the first meiotic division. Analysis for transcripts that are expressed in primary spermatogonia and preleptotene spermatocytes^{10–20} indicated that there was an upregulation of transcription of these genes in *A-myb*^{-/-} mice. Thus, the expression of *c-fos*, *c-jun*, *junB*, *c-myc*, GATA-1 and *c-kit* was increased in *A-myb*^{-/-} mice. A similar pattern of expression was seen for Krox20 and Krox24, which are early growth response genes^{13,14}. The increased expression of all these genes may reflect a compensatory feedback regulatory effect due to the lack of fully differentiated end-product cell types in these mice. Alternatively, increased expression of these genes may be due to a relative increase in the proportion of the populations of early germ cells in *A-myb*^{-/-} mice. The *ctk*¹⁵ and *Fra-1* (ref. 16) genes, which are expressed in primary spermatocytes before the pachytene stage, haploid spermatids and Leydig cells, were found to be expressed in *A-myb*^{-/-} testes, but in decreased amounts (Fig. 3b). Expression of Hox-1.4, caldesmon and proacrosin-binding genes, which are initially expressed in late-stage primary spermatocytes,^{17–19} was not observed in *A-myb*^{-/-} samples (Fig. 3a, b).

Testis-specific expression of PGK-2 and Hsp70-2 begins in leptotene and peaks in pachytene spermatocytes^{20,21}. Our analysis

Table 1 Weights of *A-myb*^{-/-} male mice and their testes at various ages compared with controls

Age (weeks)	(-/-) W (g)	(-/-) W _t (g)	(-/-) W _t /W (%)	(+/-) W (g)	(+/-) W _t (g)	(+/-) W _t /W (%)	(+/+) W (g)	(+/+) W _t (g)	(+/+) W _t /W (%)
4	8.5	0.0185	0.22	22.9	0.138	0.6			
6	17.7	0.046	0.26				23.2	0.196	0.84
10	16.9	0.06	0.35				26.9	0.158	0.80
12	21.4	0.052	0.24	29.6	0.278	0.94			

Male mice were killed at different ages. The total body weight (W) in grams of each mouse was compared with the weight of its testes (W_t). With age, the total body weight of *A-myb* mice increases to ~70% of that of wild type (+/+) and heterozygous (+/-) littermates, but *A-myb*^{-/-} testes remain at ~25–40% at the weight of their littermates.

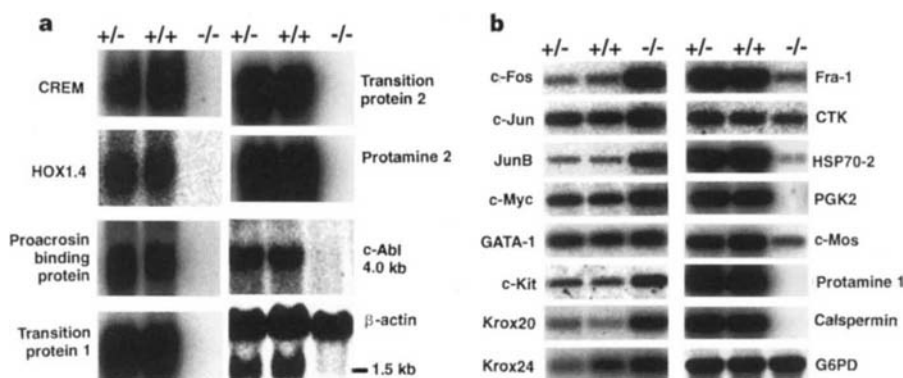


Figure 3 Analysis of gene expression in *A-myb*-deficient mice. **a**, Northern blot analysis using probes for CREM, proacrosin binding protein, *c-abl*, Hox 1.4, transition protein 1, transition protein 2, protamine 2 and actin. **b**, RT-PCR analysis of *c-fos*, *c-jun*, *junB*, *c-myc*, GATA-1, *c-kit*, krox20, krox24, *Fra-1*, CTK, HSP70-2,

PGK-2, *c-mos*, protamine 1 and caldesmon. Quantitative PT-PCR analysis was done with 1 µg total RNA, followed by Southern blot analysis using specific internal ³²P-labelled oligonucleotide probes as described⁷.

shows a complete absence of PGK-2 and very low levels of Hsp-70-2-gene transcripts in *A-myb*^{-/-} mice. Both the Hsp70-2 and PGK-2 genes contain Myb-binding sites in their promoter/enhancer domains^{20,21} and are thus good candidates for regulation by *A-myb* during spermatogenesis. It is interesting that Hsp70-2 and CREM, which are not expressed in *A-myb*^{-/-} mice, are critical in spermatogenesis, as shown in gene knockout experiments^{7,22,23}. Hsp70-2^{-/-} mice lack post-meiotic spermatids and mature spermatozoa²², a phenotype similar to that of *A-myb*^{-/-} mice. CREM^{-/-} mice, on the other hand, suffer post-meiotic arrest and lack secondary spermatids and mature spermatozoa^{7,23}. These results and histochemical studies indicate that spermatogenesis in *A-myb*^{-/-} mice may be blocked at early pachytene, immediately after leptotene.

The ovaries of the *A-myb*^{-/-} female mice appeared histologically normal (data not shown). When *A-myb*^{-/-} female mice were

mated with wild-type or *A-myb*^{+/-} mice, they became pregnant and produced litters of normal size. However, a dramatic abnormality in mammary function became apparent after *A-myb*^{-/-} female mice delivered their pups: these females were unable to nurse their pups and their mammary tissue proliferation was defective.

To determine whether *A-myb* plays a role in breast development, we examined the pattern of expression in breast tissues derived from virgin, pregnant and lactating mice. *In situ* hybridization showed that *A-myb* is not expressed, or only at very low levels, in ductal cells derived from virgin mice (Fig. 4A). These levels increase dramatically during the cell proliferation that accompanies pregnancy, resulting in ductal branching and alveolar development (Fig. 4A, h and i). As this phase of ductal branching is mainly induced by the combined action of oestrogen and progesterone^{24,25}, these results indicate that *A-myb* might be important in this phase of ductal

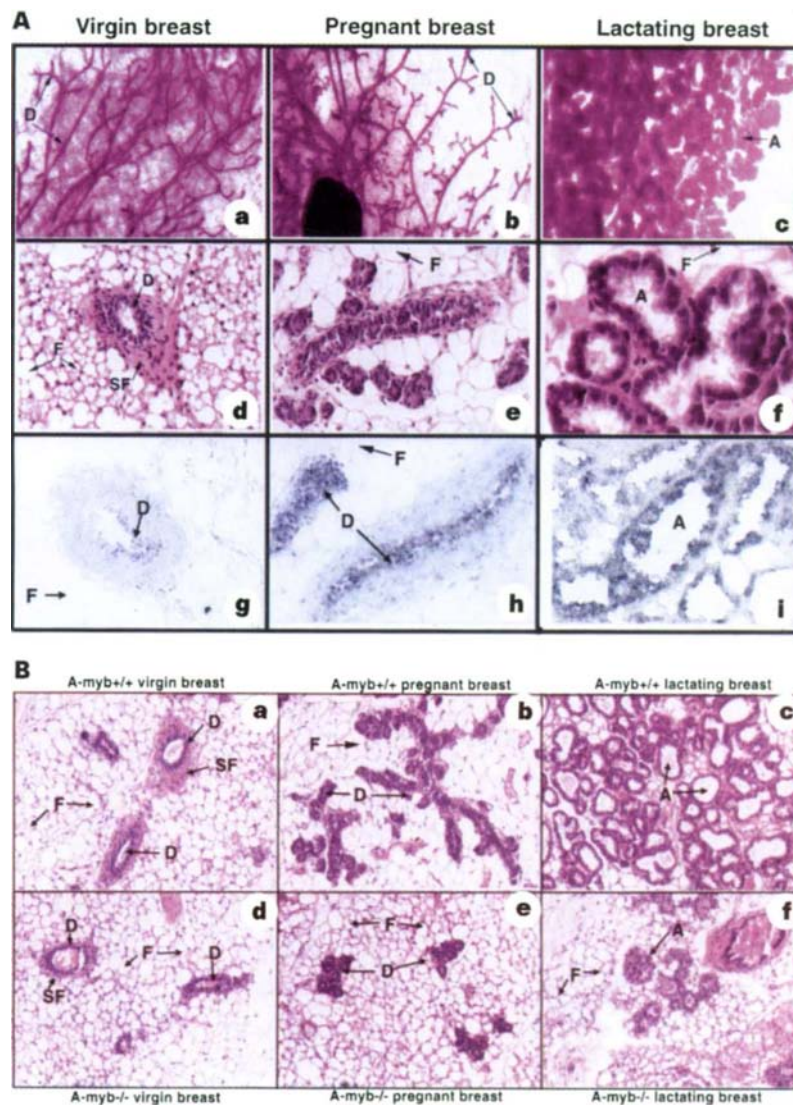


Figure 4 A, Expression of *A-myb* during pregnancy-induced ductal branching and lobulo alveolar development. Whole-mount preparations of mammary glands derived from **a**, a nulliparous wild-type mouse; **b**, 10-day pregnant wild-type mouse; and **c**, a wild-type mouse two days after delivery. **d**, **e**, **f**, Sections (magnification, 200 ×) of the same tissues stained with haematoxylin and eosin. Note the abundance of fat cells (F) in virgin and pregnant mice, and their relative absence in the lactating mouse. **g**, **h**, **i**, *In situ* hybridization of the breast tissue sections with *A-myb* specific probe². D, ductal epithelial cells; SF, fibroblasts; F, adipocytes; A, alveoli. **B**, Impaired mammary epithelial expansion during preg-

nancy of *A-myb*^{-/-} mice. Mammary glands derived from nulliparous wild-type and *A-myb*^{-/-} (**a**, **d**) mice, 10-day pregnant wild-type and *A-myb*^{-/-} mice (**b**, **e**), and wild-type and *A-myb*^{-/-} mice 2 days after delivery of pups (**c**, **f**) were used for histopathological analysis (magnification, 200 ×). Note the highly reduced proliferation of ductal cells and incompletely formed alveolar structures in *A-myb*^{-/-} mice two days after the birth of the pups. Breast sections of *A-myb*^{-/-} mice (two days after delivery) showed an abundance of fat cells (F), which are rare in the lactating wild-type mouse.

proliferation and morphogenesis into alveolar structures. *A-myb* is not expressed, or only at very low levels, in fibroblasts and adipocytes that surround the ductal cells of the breast (Fig. 4A, g-i).

Histopathological examination of the mammary epithelium revealed that the breast epithelium of *A-myb*^{+/+} and *A-myb*^{+/-} mice underwent the expected massive expansion following pregnancy (Fig. 4B, b and c), but the proliferation of mammary epithelium was considerably diminished in pregnant *A-myb*^{-/-} mice (Fig. 4B, e and f). Specifically, the amount of breast tissue was markedly decreased (by more than 60%) in post-partum *A-myb*^{-/-} mice compared to their lactating wild-type or heterozygous littermates. This appeared to be due to reduced cell proliferation resulting in diminished ductal branching following pregnancy (Fig. 4B, e). Although the breast tissue of post-partum *A-myb*^{-/-} mice contained a few structures resembling alveoli, these appeared to be incompletely formed (Fig. 4B, f). The histopathology of post-partum breast epithelium also showed the presence of considerable amounts of fat tissue in *A-myb*^{-/-} mice (Fig. 4B, f), which was almost completely replaced by alveolar structures in *A-myb*^{+/+} or *A-myb*^{+/-} mice (Fig. 4B, c). Thus, the loss of *A-myb* expression seems to result in a loss or diminution of progesterone-induced proliferative events associated with the pregnancy-induced morphogenesis of breast tissue. There appears to be progression towards a differentiated phenotype in the existing ductal tissue, but inadequate proliferation of ductal epithelium seems to result in incomplete and defective alveolar and lobular growth and development. These defects in proliferation could be a result of loss of *A-myb*, which may mediate proliferation of cells directly, or of an alteration in the levels of hormones that affect breast development.

The testis histopathology of *A-myb*^{-/-} mice is indistinguishable from that seen in testicular biopsies taken from men suffering from spermatocytic arrest^{26,27}, suggesting that defects in *A-myb* expression or function may constitute the molecular basis of this form of human infertility. In addition, as many ductal carcinomas of the breast express high levels of *A-myb* (our unpublished data), this gene may be important in the genesis of breast carcinomas. □

Methods

Gene targeting. A 5.9-kb mouse genomic fragment was cloned from a murine 129/J library that was sequenced completely and found to contain exons III, IV and V, encoding the 5' end of the DNA-binding domain of the A-Myb protein (Fig. 1a). To disrupt the DNA-binding domain of the *A-myb* gene, a targeting vector was designed in which the neomycin transferase (*NEO*) gene was inserted into exon IV at the *Clal* site (Fig. 1a). The targeting vector was introduced into ES cells by electroporation⁴. Double selection in G418 and gancyclovir produced 87 clones that were analysed by Southern blotting using a 700-bp *SspI* 5' *A-myb* probe (Fig. 1a). The appearance of an 8-kb band indicated a homologous recombination event in two of the clones analysed. The two clones bearing a disrupted *A-myb* gene were used to generate *A-myb*^{+/-} mice.

Northern blot and RT-PCR analysis. Northern blots of 20 µg total RNA were analysed. The *A-myb* probe has been described⁷. Quantitative RT-PCR analysis was done with 1 µg total RNA according to ref. 7. Primers were selected within coding sequences of each gene and the identity of each amplified band was confirmed by Southern blotting using specific internal ³²P-labelled oligonucleotide probes as described⁷. In addition, each amplified band was cloned into a plasmid vector and sequenced.

Histopathological analysis. Testis sections were prepared and stained with toluidine blue. Whole mounts of breast tissues were prepared and stained with carmine red as described²⁸. Histological sections of breast tissue were stained with haematoxylin and eosin. *In situ* hybridization analysis was done using an *A-myb*-specific probe as described.²

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Maintenance of somite borders in mice requires the *Delta* homologue *Dll1*

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During vertebrate embryonic development, the paraxial mesoderm is subdivided into metameric subunits called somites. The arrangement and cranio-caudal polarity of the somites governs the metamerism of all somite-derived tissues and spinal ganglia. Little is known about the molecular mechanisms underlying somite formation, segment polarity, maintenance of segment borders, and the interdependency of these processes. The mouse

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Delta homologue *Dll1*, a member of the DSL gene family, is expressed in the presomitic mesoderm and posterior halves of somites¹. Here we report that, in *Dll1*-deficient mouse embryos, a primary metameric pattern is established in mesoderm, and cytodifferentiation is apparently normal, but the segments have no cranio-caudal polarity, and no epithelial somites form. Caudal sclerotome halves do not condense, and the pattern of spinal ganglia and nerves is perturbed, indicating loss of segment polarity. Myoblasts span segment borders, demonstrating that these borders are not maintained. These results show that *Dll1* is involved in compartmentalization of somites, that dermo-myotome and sclerotome differentiation are independent of formation of epithelia and subdivision of somites in cranial and caudal halves, and that compartmentalization is essential for the maintenance of segment borders in paraxial mesoderm-derived structures.

Dll1 is expressed in the paraxial mesoderm suggesting that it might be involved in aspects of somite formation and patterning¹. To examine its role during embryogenesis, the *Dll1* gene was mutated by homologous recombination in embryonic stem cells such that amino acids 2–116 were replaced with an in-frame fusion of the *lacZ* gene of *Escherichia coli* (Fig. 1a). This allele, called *Dll1^{lacZ}*, was introduced into the mouse germ line. The expression of *lacZ* in the central nervous system (CNS) and paraxial mesoderm in heterozygous embryos (Fig. 2a–d) was in agreement with previous expression studies¹, and provided a versatile marker for myotome, spinal ganglia and spinal nerves in mutant embryos.

Homozygous *Dll1^{lacZ}* embryos show severe patterning defects in the paraxial mesoderm and a hyperplastic CNS. Mutants become severely haemorrhagic after embryonic day 10, and die around day 12 of development. Analysis of defects in the CNS and paraxial mesoderm suggests that *Dll1* has different biological functions in these tissues. In the CNS, loss of *Dll1* function leads to excessive neuronal differentiation, consistent with the described participation of *Delta* in cell fate decisions in *Drosophila*² and *Xenopus*³

neurogenesis. Here we analyse the patterning defects in the paraxial mesoderm, which suggest functions in mesenchymal–epithelial transitions and somite patterning.

Metamerism becomes obvious in the mesoderm of mouse embryos from around day 8 with the formation of somites. Mutant *Dll1^{lacZ}* embryos were identified from day 8.5 onwards by apparent defects in the segmentation of mesoderm. Segments were present in the trunk, but were irregularly shaped, reminiscent of the defects described in *Notch1* mutant embryos⁴, and they were absent in the tail bud of older embryos. Histological analysis of mutant embryos revealed various patterning defects in paraxial mesoderm derivatives (Figs 3 and 4). Sections of day-10.5 mutant embryos showed distinct dermatomes and dermomyotomes in the trunk. However, the normally segmented arrangement of myotome and sclerotome cells was disturbed. In wild-type embryos, each myoblast stretches over the whole width of, and is strictly confined to, one segment^{5,6}. In contrast, in homozygous *Dll1^{lacZ}* embryos, myoblasts spanned and connected adjacent segments (Fig. 3b,d), suggesting that segment borders are not maintained. In wild-type embryos, sclerotome cells condense in the caudal halves of segments, whereas cells of the cranial halves initially remain mesenchymal, resulting in a segmented pattern of densely and loosely packed cells ventral to the spinal cord (Fig. 3e). In mutant embryos, sclerotome cells formed a uniform, loose mesenchyme without a segmented pattern (Fig. 3f). The condensations in caudal segment halves were apparently missing, suggesting that the identity of caudal segment halves was lost. This interpretation was supported by the occurrence of fused ganglia, and wide and irregularly spaced

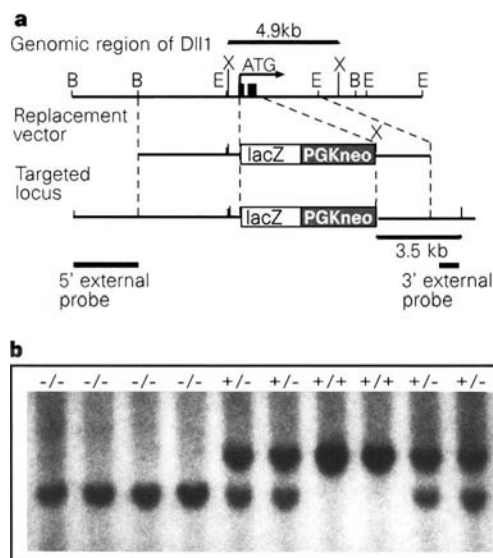


Figure 1 Generation of mice carrying a germline mutation of *Dll1*. **a**, Restriction maps of mouse *Dll1* genomic DNA, the replacement vector for gene targeting, and predicted structure of the *Dll1^{lacZ}* allele after homologous recombination. The replaced exons are indicated as black boxes. Restriction sites: B, *Bam*HI; E, *Eco*RI; X, *Xba*I. Sizes of DNA fragments from the wild-type and mutated allele detected by the 3' external probe used in **b** are indicated. **b**, Southern blot analysis of day-10 embryos derived from matings between heterozygous *Dll1^{lacZ}* mice. The 4.9-kb and 3.5-kb *Xba*I fragment corresponding to the wild-type and the targeted allele, respectively, were identified with the 3' external probe shown in **a**. +/+, wild type; +/-, heterozygote; -/-, homozygote.

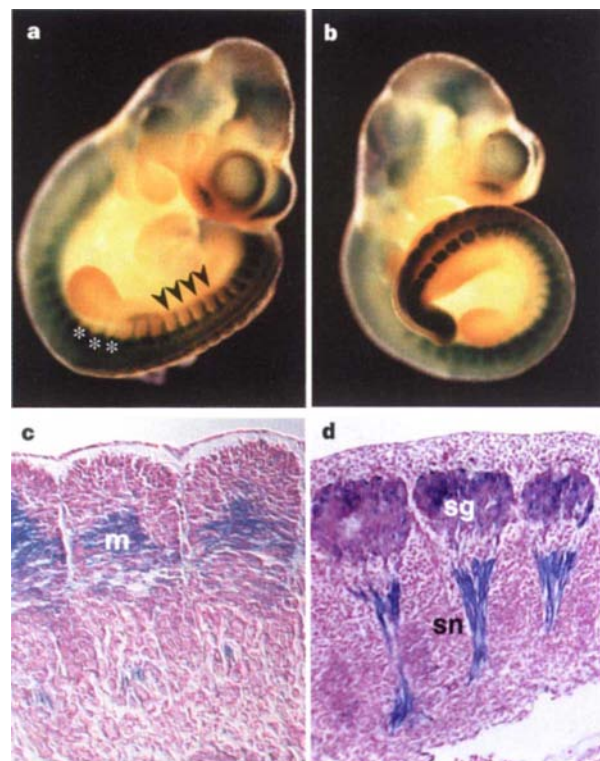


Figure 2 β -galactosidase staining in heterozygous *Dll1^{lacZ}* embryos. β -galactosidase staining was detected throughout the CNS (**a**, **b**), in the presomitic mesoderm (**b**), posterior halves of condensed somites (arrowheads in **a**), myotome (**c**) and spinal ganglia and spinal nerves (**d**, and asterisks in **a**). Abbreviations: m, myotome; sg, spinal ganglion; sn, spinal nerve.

spinal nerves in homozygous *Dll1^{lacZ}* embryos (Fig. 3h, j) rather like the defects obtained in experimentally produced multiple cranial compound somites in chick embryos⁷. Normally, spinal ganglia and nerves form only in the cranial halves of segments, owing to factors inhibitory for neural-crest cell migration and axonal growth in caudal segment halves^{8–12}.

Although the dermomyotome showed clear evidence of segmental subdivision in the trunk of mutant day-10.5 embryos, no segmentation was observed in the tail bud, and no epithelial somites were found (Fig. 4b), whereas tail buds of wild-type embryos contain several clearly discernible somites (Fig. 4a). To investigate whether the lack of epithelial somites is specific for the late tail bud, serial sections of mutant day 9–9.5 embryos were analysed. In these embryos the posteriormost segments correspond to the lower thoracic and lumbar levels. As in the tail buds of day-10.5 embryos, no epithelial somites were found in mutant day-9 embryos (*N* = 15). Instead, the presomitic mesoderm at its anterior end gradually gained the appearance of an unsegmented dermomyotome with associated mesenchymal cells (Fig. 4d), preceded anteriorly by clearly segmented dermomyotomes.

To define more precisely the patterning defects in mesoderm, we

analysed the expression of various marker genes between days 9 and 10.5 of development (Fig. 5, and data not shown). The analysis of markers for dermomyotome and sclerotome (*paraxis*; ref. 13), myotome (*myogenin* and *myf5*; ref. 14) and sclerotome (*Pax9*; ref. 15) confirmed that the cellular differentiation products of somites were present in mutant embryos despite the absence of epithelial somites. However, consistent with the histological findings, the segmented expression of the analysed genes was perturbed or abolished. Both *myogenin* and *myf5* maintained a segmented pattern of expression. However, the stripes of cells expressing *myogenin* and *myf5* were no longer evenly spaced, and frequently merged (Fig. 5b, d), consistent with the fused myotomes seen in histological sections (Fig. 3b, d). In contrast to wild-type embryos, which show clearly segmented patterns of *Pax9* and *Pax1* expression¹⁶ (Fig. 5e, and data not shown), expression in mutant embryos was diffuse, without the strong expression in the posterior segment halves (Fig. 5f). Similarly, *paraxis* expression was irregular and diffuse in mutant embryos (Fig. 5h). *Mox1* is expressed in all cell types of somites¹⁷, exhibiting a domain of weak expression in the anterior portion (arrows in Fig. 5i) and a domain of strong expression in the posterior portion of somites (arrowheads in Fig. 5i) in wild-type embryos. In mutant embryos, *Mox1* transcripts were no longer differentially expressed in the anterior and posterior regions of somites, but seemed to be present at similar levels (arrows in Fig. 5j). Thus the expression patterns of *Pax9*, *paraxis* and *Mox1* were altered in *Dll1* mutant embryos, consistent with the histological observation that the cranio-caudal polarity of segments is disrupted in embryos lacking *Dll1*. As a marker for axial development, expression of *Sonic hedgehog*¹⁸ was analysed. No differences of *Shh* expression in the tail buds were detected between wild-type and mutant embryos, suggesting that axis formation and elongation is largely normal in mutant embryos (data not shown).

Our analysis indicates that *Dll1* is required for the formation of epithelial somites, but that epithelial somites are not essential for establishing a metamereric pattern, or for the differentiation of the dermomyotome and sclerotome. Similar to the requirement for *Delta*-mediated cell-to-cell interactions among cells that acquire or maintain an epithelial phenotype in *Drosophila*¹⁹, *Dll1* might be required for the mesenchymal–epithelial transition during somitogenesis. The loss of cranio-caudal polarity in somites could be secondary to the defect in epithelialization, or both phenotypes

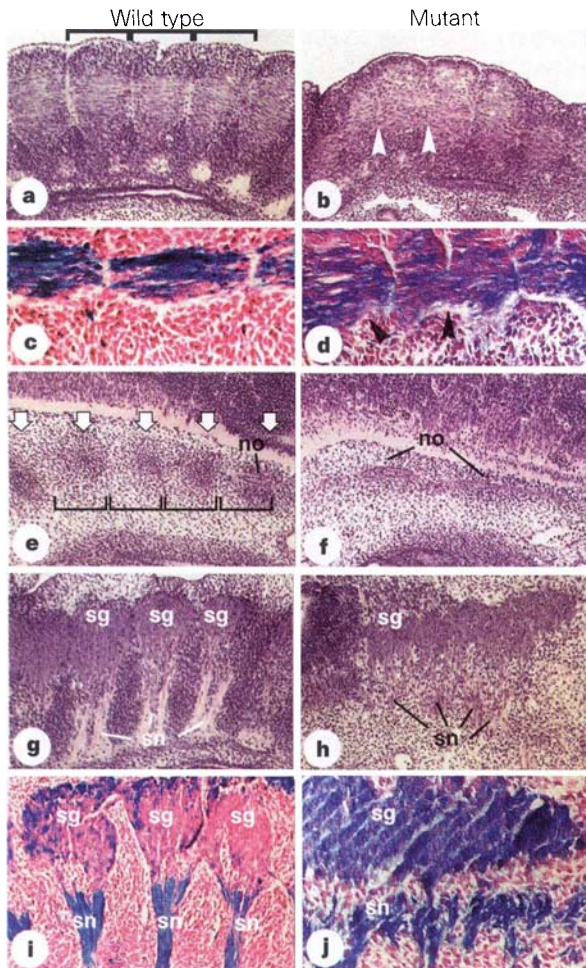


Figure 3 Patterning defects in homozygous day-10.5 *Dll1^{lacZ}* mutant embryos. In contrast to wild-type (a) and heterozygous (c) embryos, in mutants (b, d) myoblasts bridged and connected adjacent segments (arrowheads). Alternating areas of dense and loose sclerotome were found in wild type (e), but only uniformly loose mesenchyme, without signs of metamereric condensations, was found in mutant embryos (f). Spinal ganglia and nerves were evenly spaced and distinct in wild-type (g) and heterozygous (i) embryos, but were fused and irregular in mutants (h, j). Brackets in wild-type embryos indicate segmental units. All sections are sagittal; no, notochord; sg, spinal ganglion; sn, spinal nerve.

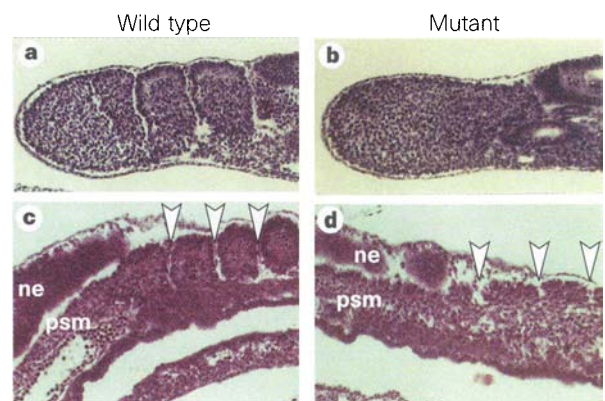


Figure 4 Absence of epithelial somites. Epithelial somites were present in the tail tip of day-10.5 wild-type embryos (a) but absent in mutants (b). Day-9.5 wild-type embryos contained well-defined epithelial somites anterior to the presomitic mesoderm (c). In mutants (d), anterior to the presomitic mesoderm, segmented dermomyotome-like structures were present, but there were no epithelial somites. Segment borders are indicated by arrowheads. Posterior is to the left. All sections are sagittal; ne, neuroectoderm; psm, presomitic mesoderm.

could result independently as consequences of loss of *Dll1* function. Expression of *Dll1* in the presomitic mesoderm and posterior halves of epithelial somites is compatible with roles of *Dll1* in epithelialization and compartmentalization. Several studies suggest that both processes are independent. In chick embryos, the cranio-caudal polarity of segments is established before the formation of the epithelial somite^{5,8}, suggesting that epithelial somites are not required for the generation of cranial and caudal compartments. In mouse embryos that lacked the bHLH protein Paraxis, no epithelia formed in the paraxial mesoderm²⁰, but no defects in the cranio-caudal polarity of somites were noted. Thus the loss of compartmentalization in *Dll1* mutant embryos is probably not secondary to the epithelialization defect. Expression of *paraxis* is altered and reduced in *Dll1* mutant embryos (Fig. 5h), raising the

possibility that *Dll1*-mediated signals regulate *paraxis* expression. In this case, the defects in somite epithelialization might develop as a consequence of impaired Paraxis function. However, the epithelialization defects in homozygous *Dll1*^{lacZ} embryos are much milder than in *paraxis* mutants.

Our results provide strong genetic evidence that the polarity of somites is required for maintaining segment borders, as has been proposed previously⁷ on the basis of experimental studies in chick embryos. Those studies showed that when like somite halves (cranial with cranial, or caudal with caudal) were combined, cells mixed and no borders formed, whereas abutting cells from unlike somite halves resulted in formation of a border⁷. Because confronting anterior with posterior somite compartments is sufficient for border formation, and loss of detectable anterior–posterior polarity in somites of *Dll1*^{lacZ} mutant embryos is accompanied by loss of segment borders, it is highly likely that the polarity of somites is required for maintenance of the segmental arrangement of differentiating somitic cells. It is not clear, however, whether the initial generation of segment borders in mutant embryos occurred in the absence of cranio-caudal polarity, because we cannot exclude the possibility that segment polarity was initially established in the presomitic mesoderm of *Dll1*^{lacZ} mutants and was lost after the formation of distinct segmental somites. Based on theoretical considerations, it has been suggested²¹ that the interaction between the different cranial and caudal compartments could be a mechanism to form segment (somite) borders. Alternatively, the primary metamereric pattern could be established by a system with a two-segment periodicity analogous to the action of pair-rule genes in *Drosophila*, and the primary segments are subsequently subdivided into anterior and posterior compartments²¹. Once appropriate markers are available to determine whether or not cranial and caudal compartments are established initially in the presomitic mesoderm of *Dll1* mutants, further analysis of *Dll1* mutant embryos might provide experimental evidence in support of one of these models for segmentation in vertebrates. □

Methods

Generation of mice carrying a germline mutation of *Dll1*. Mouse *Dll1* genomic clones were isolated from a strain 129/Sv^{Pas} library (provided by K. Schuster-Gossler) and analysed using standard protocols²². The replacement vector was constructed by inserting a 4-kilobase *Clal/XhoI* fragment, which encodes a part of the β -galactosidase gene followed by the *PGK-neo* cassette, and a 2.6-kb *XhoI/EcoRI* fragment as the 3' homologous region of the replacement vector into pKS+. This vector was linearized with *SalI* and *Clal*, and a 4.1-kb *SalI/NcoI* as the 5' homologous region plus an *NcoI/Clal* fragment (800 base pairs) completing the β -galactosidase gene, were inserted, resulting in the complete replacement vector. This vector was linearized with *SalI* and electroporated into R1 embryonic stem cells²³. Correctly targeted clones were identified and verified by Southern blot analysis using external probes from the 3' and 5' region of the targeted area, and were injected into C57BL/6J embryos to obtain germline transmission.

Analysis of mutant embryos. Noon of the day a vaginal plug was observed and counted as day 0.5 of gestation. Embryos were initially genotyped by Southern blot hybridizations, or allele-specific polymerase chain reaction (PCR) of yolk-sac DNA using the primers *Dll1*/38 ATCCCTGGGTCTTTGAA-GAAG (which binds just upstream of the *Dll1* translation initiation codon) and *Dll1*/39 ATACGCGAAGAAGGTCCTG (which binds in exon 2), which detect a 482-bp fragment indicative for the wild-type allele, and primers *Dll1*/38 and *lacZ*1 ATCCTCTGCATGGTCAGGTC, which detect a 578-bp fragment indicative of the mutated allele. After genotyping 22 mutant embryos we were convinced that homozygous embryos could be identified unambiguously by the disturbed segmentation pattern, and beginning at day 10 by their additional haemorrhagic phenotype. Thus mutant embryos were subsequently identified by these criteria. For histological analyses, embryos were dissected, fixed in Bouin's fixative overnight and embedded in paraffin. Serial sections (5 μ m) were counterstained with haematoxylin and eosin, or nuclear fast red. Whole-mount *in situ* hybridizations were done as described²⁴.

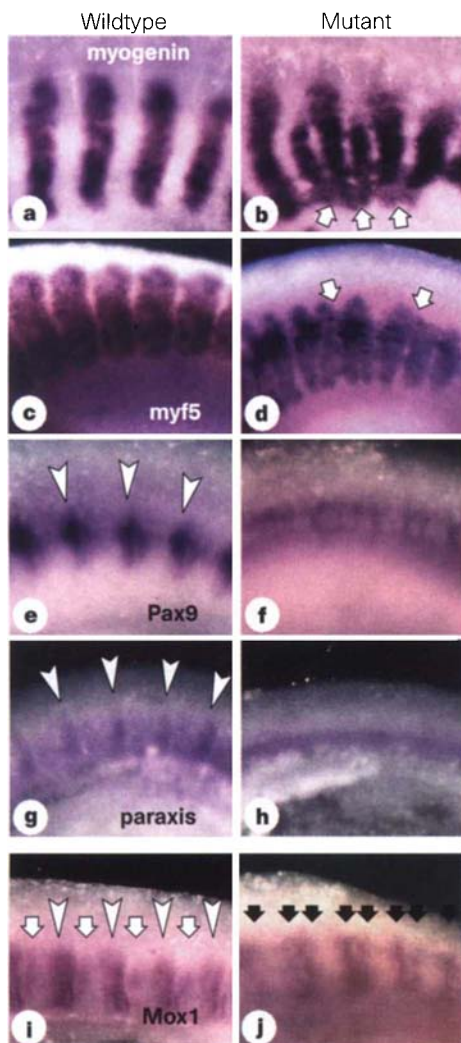


Figure 5 Altered gene expression patterns in mutant *Dll1*^{lacZ} embryos. In day-10.5 mutants, stripes of *myogenin*- and *myf5*-expressing cells were not evenly spaced, and were frequently merged (arrows in **b** and **d**). Mutants (**f**) lack the regions of strong *Pax9* expression in the posterior parts of wild-type sclerotomes¹⁵ (arrowheads in **e**). In day-9.5 wild-type embryos, *paraxis* expression reflected the segmented arrangement of somites and dermomyotomes (**g**), whereas in mutants (**h**) expression was weak and domains were ill defined and diffuse. In day-10.5 embryos, *Mox1* was differentially expressed in somites of wild-type embryos (**i**) with a domain of strong expression in posterior somite halves (arrowheads) and a domain of weak expression in anterior somite halves (arrows). In mutant embryos (**j**), stronger *Mox1* expression in the posterior parts of segments was no longer detected, instead, similar low levels of transcripts were found in the anterior and posterior regions of each segment (arrows).

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NMDA-receptor regulation of substance P release from primary afferent nociceptors

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Severe or prolonged tissue or nerve injury can induce hyperexcitability of dorsal horn neurons of the spinal cord, resulting in persistent pain, an exacerbated response to noxious stimuli (hyperalgesia), and a lowered pain threshold (allodynia). These changes are mediated by NMDA (N-methyl-D-aspartate)-type

glutamate receptors in the spinal cord¹. Here we report that activation of the NMDA receptor causes release of substance P, a peptide neurotransmitter made by small-diameter, primary, sensory 'pain' fibres. Injection of NMDA in the cerebrospinal fluid of the rat spinal cord mimicked the changes that occur with persistent injury², and produced not only pain, but also a large-scale internalization of the substance P receptor into dorsal horn neurons, as well as structural changes in their dendrites. Both the pain and the morphological changes produced by NMDA were significantly reduced by substance P-receptor antagonists or by elimination of substance P-containing primary afferent fibres with the neurotoxin capsaicin. We suggest that presynaptic NMDA receptors located on the terminals of small-diameter pain fibres facilitate and prolong the transmission of nociceptive messages, through the release of substance P and glutamate. Therapies directed at the presynaptic NMDA receptor could therefore ameliorate injury-evoked persistent pain states.

Behavioural and electrophysiological studies have provided evidence that substance P and glutamate interact synergistically in the excitation of dorsal-horn nociceptive neurons and in the production of pain^{3,4}. Because NMDA receptors are expressed abundantly on dorsal horn neurons^{5,6} the interaction between substance P and glutamate was presumed to occur postsynaptically, on the cell bodies and dendrites of dorsal horn neurons that express substance P and NMDA receptors. This mechanism would be comparable to that proposed for long-term potentiation in the CA1 region of the hippocampus, where only postsynaptic NMDA receptors are found⁷. In the spinal cord, however, NMDA receptors are also expressed presynaptically, on the terminals of substance P-containing primary afferent fibres^{8,9}. We now provide evidence that activation of these presynaptic receptors enhances the postsynaptic effects exerted by glutamate in the production of pain.

Intrathecal injection of NMDA in the rat, as in the mouse², produced pain behaviour consisting of caudally directed biting, licking and scratching. The behaviour appeared within seconds of injection and lasted more than 2 min. Prior injection of AP5, a selective NMDA antagonist, completely blocked the pain behaviour, whereas CNQX, an AMPA receptor-selective antagonist had no effect. However, prior intrathecal injection of a selective antagonist of substance P receptors, CP-99,994 (Merck) or RP-67,580

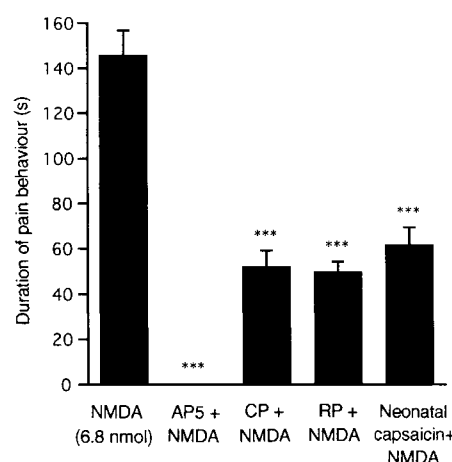


Figure 1 To determine the receptor through which NMDA exerted its effects, we used a dose of 6.8 nmol, which produced consistent pain behaviour without motor side effects. The pain behaviour, which appears within 15 s and lasts 2.5 min, was blocked by prior (10 min) injection of the NMDA antagonist AP5 (15 nmol), and was significantly reduced by injection of substance P-receptor antagonists (CP-99,994, 0.75 μ M; or RP-67,580, 20 nmol). There was a comparable reduction of pain behaviour in rats treated neonatally with the C-fibre neurotoxin capsaicin.

(Rhone-Poulenc), significantly reduced the duration of NMDA-induced pain behaviour. This result raised the possibility that the pain behaviour produced by NMDA is generated in part by release of substance P from primary afferent C ('pain') fibres. Consistent with this hypothesis, neonatal treatment with the neurotoxin capsaicin, which destroys NMDA receptor-laden C fibres, also significantly reduced the pain behaviour produced by NMDA (Fig. 1).

We have previously demonstrated that the central consequences of substance P release can be assessed by quantifying the internalization of the substance P receptor in subpopulations of dorsal horn neurons^{10,11}. Here we used electron microscopic immunocytochemistry to analyse internalization of substance P receptors in spinal cord tissue collected from the NMDA-treated rats. In

unstimulated rats, 73% of the receptors in lamina I neurons are located on the plasma membrane. After NMDA injection, there is a profound internalization of these receptors in neurons of the lumbar enlargement, comparable to that produced by intrathecal injection of substance P or noxious stimulation^{10,11}. Five minutes after NMDA injection, which corresponds to the period during which we recorded pain behaviour, over 80% of the substance P receptors were located in the cytoplasm (Fig. 2); this process was reversible. By 60 min, most (68%) of these receptors were again located on the plasma membrane, and by 24 h their distribution was the same as in untreated tissue. Because 60 min is too short a period for new synthesis to have occurred, it is likely that recycling of the internalized substance P receptors had occurred. Although the

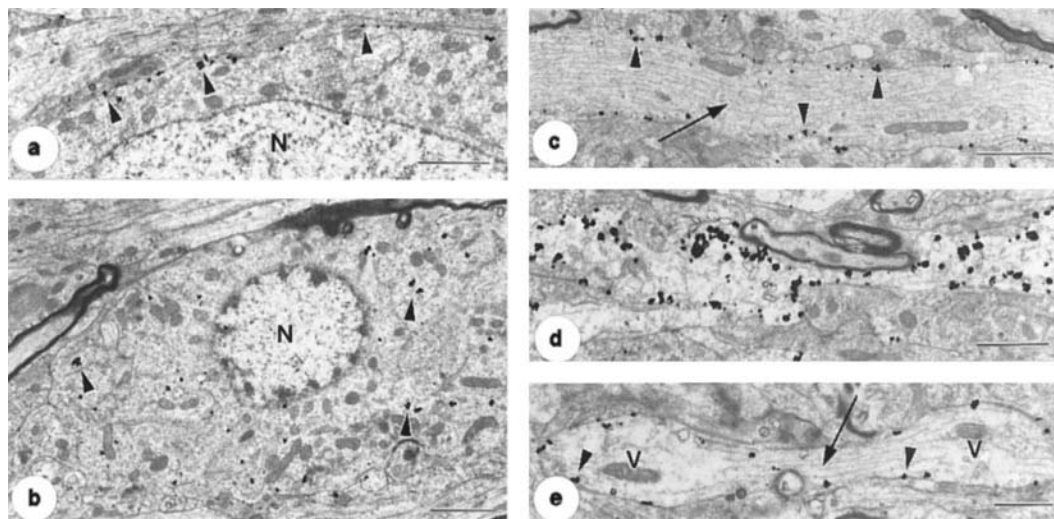


Figure 2 In untreated rats, the substance P receptor is concentrated over the plasma membrane of lamina I cell bodies (arrowheads in **a**) and dendrites (arrowheads in **c**). Within 5 min of NMDA injection, over 80% of the substance P-receptor labelling is located in the cytoplasm of cell bodies (arrowheads in **b**) and in the varicosities of structurally altered dendrites (**d**). Microtubules are present in normal dendrites (arrow in **c**) and in the intervaricose segments of altered

dendrites (arrow in **e**), but not in the varicosities (**d** and **e**). Within 60 min of NMDA injection, substance P-receptors are again concentrated on the plasma membrane. The presence of some varicose dendrites (**v** in **e**) with only plasma-membrane substance P receptors (arrowheads) suggests that recycling of the receptors precedes normalization of dendrite structure. Scale bars: 1.0 μm in **a**, **c**, **d** and **e**, 1.5 μm in **b**.

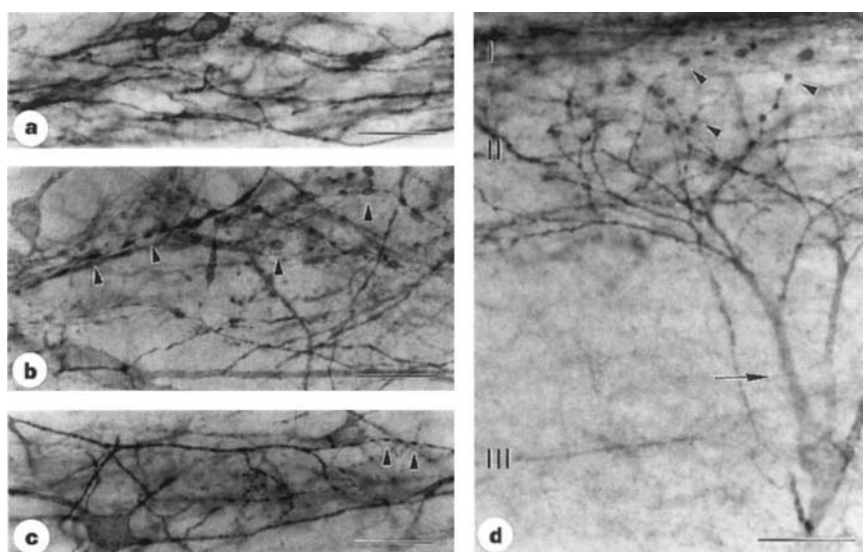


Figure 3 In untreated rats, the dendrites of lamina I neurons are smooth and arborize rostrocaudally within lamina I (**a**). After NMDA injection, the dendrites, here viewed in sagittal section, became highly varicose (arrowheads in **b**). Varicosities also appeared in the most dorsal part of the dendrites of lamina III neurons (arrowheads in **d**), but we did not find comparable changes in the lamina

III cell body (arrow in **d**). Prior injection of a substance P-receptor antagonist, RP-67580, significantly reduced the incidence of dendrite restructuring (**c**). These photomicrographs were scanned, digitized and the contrast enhanced so that the varicosities could be better illustrated. Scale bars: 20 μm in **a**, **b** and **c**, 10 μm in **d**.

membrane may have been repopulated by pre-existing receptors that were 'stored' in the cytoplasm, these would have to have been receptors not initially recognized by the antiserum.

NMDA treatment also induced a dramatic structural change in the dendrites of neurons that express substance P receptors (Figs 2 and 3). The changes were concentrated in regions targeted by primary afferent fibres containing substance P, including dendrites of lamina I neurons and the dorsally arborizing dendrites of neurons located more ventrally, in lamina III (Fig. 2d). In untreated rats, most of the dendrites are smooth (Figs 2c, 3a and 4), but within 5 min of NMDA injection the dendrites developed numerous varicosities along their length that were packed with internalized substance P receptors. By 60 min, most of the dendrites had regained their smooth architecture. We occasionally observed dendrites with varicosities at 60 min, but even in these the receptor had largely been recycled to the plasma membrane (Fig. 2d). These observations suggest that recycling of the receptor to the surface occurs before the structural changes are resolved; whether the initial internalization of the receptor drives the structural change is unknown.

AP5, but not CNQX, completely blocked both the internalization of substance P receptors and the morphological changes produced by NMDA injection. More importantly, co-injection of NMDA with an antagonist to substance P receptors significantly reduced both the magnitude of receptor internalization and the dendritic structural changes, at the same doses that reduced the NMDA-evoked pain behaviour (Fig. 3c and 4). Finally, just as neonatal treatment of rats with capsaicin significantly reduced the pain behaviour produced by intrathecal injection of NMDA (Fig. 1), so there was a significant reduction in the magnitude of internalization and dendritic structural changes (Fig. 4). However, a substantial behavioural response to NMDA persisted in the capsaicin-treated rats.

These results indicate that release of substance P contributes to the behavioural and morphological changes produced by NMDA. Two testable predictions follow from these results. First, if two intrathecal injections of NMDA are made 5 min apart, the pain behaviour produced by the second should be less than the first, because the first injection reduced the amount of substance P receptor 'available' to the substance P release by the second injection. On testing this prediction we found that the response to the second injection of NMDA was indeed significantly reduced, by 40% ($P < 0.05$, comparing the pain behaviour induced by the first and second injections). The residual response presumably reflects the pain behaviour that is mediated by the interaction of NMDA with postsynaptic receptors. Second, because the pain behaviour produced by intrathecal substance P injection almost certainly involves the same receptors that undergo internalization after intrathecal NMDA injection, we predicted that intrathecal NMDA-evoked internalization of the substance P receptors would reduce the pain behaviour produced by a subsequent injection of substance P. We recorded a $>90\%$ decrease in the pain behaviour produced by substance P (10 nM) when the injection was made 5 min after a previous NMDA injection ($P < 0.003$, comparing the pain behaviour induced by the first and second injections). The duration and intensity of the pain behaviour produced by substance P are less than that produced by NMDA, which emphasizes that the substance P released in response to NMDA injection is not the exclusive mediator of the pain behaviour produced by NMDA. Rather, our results indicate that the release of substance P exacerbates and prolongs the pain behaviour generated when NMDA interacts with postsynaptic receptors. This is consistent with evidence that substance P greatly enhances the discharge of dorsal horn neurons in response to NMDA^{3,4}.

The presence of presynaptic NMDA receptors, taken together with the results from rats treated neonatally with capsaicin, which destroys primary afferent fibres, strongly suggests that the morpho-

logical and behavioural effects produced by intrathecal NMDA injection involve the release of substance P from small-diameter primary afferent fibres. We cannot exclude the possibility that NMDA also acts on postsynaptic receptors, causing release of a diffusible retrograde messenger, such as nitric oxide, which then induces release of substance P from presynaptic terminals¹². Moreover, because there was a significant residual response in the capsaicin-treated rats, it is conceivable that NMDA also evokes the release of substance P from interneurons in the dorsal horn¹³.

The same circuit that is engaged by intrathecal injection of NMDA would contribute to the hyperalgesia, allodynia and persistent pain produced by activity of the primary afferent fibre evoked by injury to tissue or nerve. Importantly, substance P and glutamate are found in the same primary afferent terminal¹⁴. Thus glutamate released from C-fibres following tissue or nerve injury would not only depolarize postsynaptic neurons, but could also act on presynaptic NMDA autoreceptors to maintain the depolarization of the presynaptic terminal, increasing intracellular Ca^{2+} (refs 15, 16) and prolonging the release and postsynaptic effects of substance P, and possibly further enhancing the release of glutamate. Taken together with the fact that persistent injury produces continuous afferent stimulation, this circuit would provide a prolonged pool of glutamate that could influence the primary afferent terminal. This circuit may also contribute to the phenomenon of wind-up, which is an

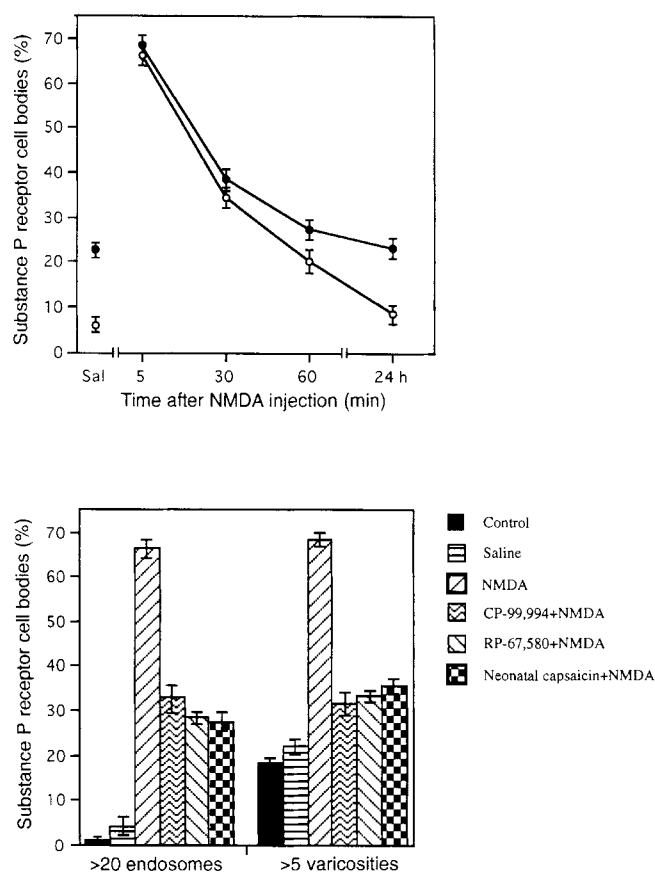


Figure 4 Top, in untreated rats, less than 1% of the substance P-receptor lamina I neurons contain more than 20 receptor-labelled endosomes, the threshold for demonstrating internalization. Within 5 min of NMDA injection (6.8 nmol; 5 min, $N = 5$; 30 min, $N = 3$; 24 h, $N = 3$), almost 67% reached threshold (open circles). In untreated rats ~20% of the lamina I dendrites contained more than 5 varicosities; these were of small diameter ($1.83 \pm 0.02 \mu\text{m}$). After NMDA injection, almost 70% of the dendrites had more than 5 varicosities (filled circles), and these were much larger ($4.5 \pm 0.14 \mu\text{m}$). Bottom, substance P-receptor antagonists, or neonatal capsaicin, significantly reduced these changes.

NMDA-mediated enhancement of the discharge of dorsal horn neurons and pain behaviour produced by repetitive C-fibre stimulation^{17,18}. Conceivably, wind-up and the prolonged hyper-excitability that it induces¹⁹ result from an NMDA receptor-mediated increase in the amount of transmitter released from the primary afferent terminal; thus each stimulus evokes a progressively greater discharge of postsynaptic neurons.

Our results indicate that presynaptic NMDA autoreceptors are part of a positive-feedback network that influences the primary afferent terminal, in a manner similar to other facilitatory autoreceptors^{20–22}. Noxious stimulation, however, also evokes the local release of endogenous opioids^{23,24}, which act presynaptically to reduce the release of substance P²⁵ and glutamate²⁶. This indicates that the balance of NMDA-evoked facilitation and opioid-mediated inhibition of substance P and glutamate release from primary afferents may critically determine the extent to which noxious stimulation produces wind-up and the persistent pain that can often occur after tissue and nerve injury. □

Methods

Behavioural analysis. For intrathecal injections we implanted rats with chronic indwelling catheters²⁷, the rats were tested 4–6 days later. Drugs were administered in doses of 10 µl followed by a 10 µl saline flush. To destroy C fibres, rats were injected subcutaneously on the first day after birth with 100 mg per kg capsaicin (Sigma) in a 1:1 Tween 80: ethanol solution (2.0 µl g⁻¹); the rats were tested 3–4 months later. The total time within 5 min for NMDA or substance P injection that the rats licked or scratched the caudal part of the body was taken as the duration of pain behaviour.

Immunocytochemistry. The rats were anaesthetized with pentobarbital (100 mg per kg) and perfused intracardially with 0.1 M phosphate-buffered 4% formaldehyde (for light microscopy) or 2% formaldehyde and 2% glutaraldehyde for electron microscopy. Sagittal vibratome sections 50 µm thick through the lumbar enlargement were incubated in a rabbit anti-substance P receptor antiserum²⁸. For light microscopy (Fig. 3) we localized the antiserum with the ABC method, with DAB and nickel enhancement. For electron microscopy we used a pre-embedding immunogold protocol in which the primary antiserum is localized with a 1.0 nM colloidal gold-labelled secondary antibody followed by silver intensification¹⁰. To quantify the distribution of substance P-receptor immunoreactivity we counted the total number of silver-enhanced particles in the plasma membrane and cytoplasm.

Quantitative analysis of internalization. For this light microscopic analysis we used 50-µm thick sections. All cell bodies recorded (average 21 per rat) and dendrites (average 389 per rat) were located within lamina I of the L4 and L5 spinal segments. A cell body with more than 20 substance P receptor-immunoreactive endosomes was considered to have internalized the receptor¹¹. Substance P receptor-immunoreactive dendrites that had at least 5 varicosities connected by thin intervaricose segments were counted. In rats treated with NMDA, over 80% of the varicosities were greater than 3.0 µm in diameter, a size not recorded in untreated tissue. The diameters of varicosities were measured after digitizing with NIH Image. Control refers to untreated rats. All slides were coded and read by an investigator blind to the treatment. Data are expressed as the means ± s.e.m. Statistical analysis of the data was performed using ANOVA and *post hoc* comparisons with Fisher's protected least significant difference.

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Induction of sodium channel clustering by oligodendrocytes

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As oligodendrocytes wrap axons of the central nervous system (CNS) with insulating myelin sheaths, sodium channels that are initially continuously distributed along axons become segregated into regularly spaced gaps in the myelin called nodes of Ranvier¹. It is not known whether the regular spacing of nodes results from regularly spaced glial contacts or is instead intrinsically specified

by the axonal cytoskeleton. Contact with Schwann cells induces clustering of sodium channels along the axons of peripheral neurons *in vitro* and *in vivo*²⁻⁴. Similarly, it has been suggested that astrocyte contact induces clustering of sodium channels along CNS axons^{5,6}. Here we show that oligodendrocytes are necessary for clustering of sodium channels *in vitro* and *in vivo*. The induction, but not the maintenance, of sodium-channel clustering along the axons of highly purified rat retinal ganglion cells in culture depends on a protein secreted by oligodendrocytes. Surprisingly, the oligodendrocyte-induced clusters are regularly spaced at the predicted interval in the absence of glial-axonal contact. Mutant rats that are deficient in oligodendrocytes develop few axonal sodium channel clusters *in vivo*. These results demonstrate a crucial role for oligodendrocytes in inducing clustering of sodium channels.

To determine whether sodium-channel clusters form on CNS axons in the absence of glia, purified retinal ganglion cells (RGCs) from rats at postnatal day 8 (P8) were cultured in serum-free medium for 2.5 weeks, a time when most nodes would have formed *in vivo*^{7,8}. Diffuse sodium-channel immunoreactivity was present along the length of most axons, but few axonal clusters were observed (Fig. 1a). Many initial segments of axons stained strongly; however, control experiments showed that these initial segments were present immediately after cell isolation, and thus had formed *in vivo*. In contrast, when RGCs were cultured either directly among or suspended over a conditioning layer of optic nerve glia, many clusters of sodium channels formed along axons (Fig. 1b). All immunoreactivity was eliminated by preabsorption with the immunizing peptide (data not shown). Under identical culture conditions, immunoreactivity of other axonal membrane proteins, including the glutamate receptor GluR6 (ref. 9), L1 (ref. 10 and Thy1.1 (ref. 11), was diffuse and not clustered (data not shown). Clusters also formed when the neurons were cultured in either glial-conditioned medium or optic-nerve extract (63 ± 9 and 88 ± 18 clusters per 100 cells (mean $\pm$ s.e.m.), respectively). These results demonstrate that the glial-derived cluster-inducing activity is soluble. Most of this activity was eliminated by boiling or trypsin treatment and

had a relative molecular mass of greater than 10,000 ($M_r > 10K$), indicating that the cluster-inducing activity is a protein.

In the CNS, sodium-channel clusters occur at axonal intervals of about 100 times the axon diameter¹². We found that the axonal sodium-channel clusters that formed in our co-cultures in the absence of direct glial-axonal contact were regularly spaced with an average intercluster distance of $44 \pm 2 \mu\text{m}$ ($N = 209$). This value is close to the predicted intercluster interval for unmyelinated RGC axons, which have a diameter of about $0.4 \mu\text{m}$ (ref. 13). The average length of sodium-channel clusters *in vitro* was similar to that of clusters seen in P10 optic-nerve cryosections (3.9 ± 0.2 and $2.6 \pm 0.1 \mu\text{m}$, respectively; $N = 100$ for each). In addition, immunoreactivity for ankyrin-G, a cytoskeletal protein that binds to sodium channels and is localized to nodes of Ranvier *in vivo*¹⁴, colocalized with axonal sodium-channel immunoreactivity in the absence (Fig. 1c) and presence (Fig. 1d) of glial cells. Together, these observations strongly suggest that *in vitro* sodium-channel clusters are structural counterparts to *in vivo* nodes of Ranvier.

To determine which glial cell type is responsible for the induction of sodium-channel clusters, RGCs were cultured over conditioning layers of either highly purified optic-nerve oligodendrocytes or astrocytes^{7,15}. Astrocytes induced little clustering, whereas oligodendrocytes induced as many clusters as mixed optic-nerve glia (Fig. 2). Although oligodendrocytes induced clusters, they were not required to maintain the clusters; induced clusters persisted at the same density two weeks after the removal of oligodendrocytes and replacement of medium that had not been preconditioned (72 ± 3 clusters per 100 cells at the time of removal; 83 ± 3 clusters per 100 cells two weeks after removal).

If oligodendrocytes are responsible for inducing clusters of sodium channels *in vivo*, the time course of the development of clusters should correlate with the development of oligodendrocytes, rather than with the development of astrocytes. Most oligodendrocytes develop after P7, whereas most astrocytes develop before this time¹⁶. We stained optic-nerve cryosections from P4, P10 and P16 rats (Fig. 3). At P4, a time before the development of oligodendrocytes, sodium-channel clusters were not detected, although

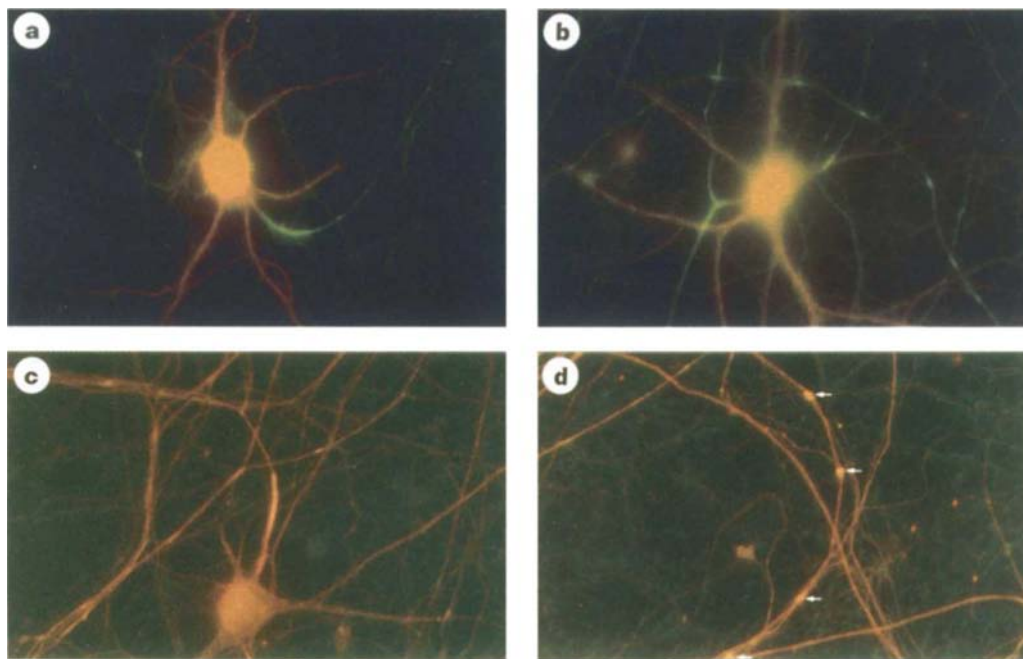


Figure 1 Sodium-channel MAP2 (**a**, **b**) and sodium-channel ankyrin-G (**c**, **d**) immunoreactivity along RGC axons cultured in the presence (**b**, **d**) or absence (**a**, **c**) of optic-nerve glia. MAP2 staining was visualized with a Texas red-conjugated anti-mouse IgG antibody (Jackson). Sodium-channel staining was

visualized with a FITC-conjugated anti-rabbit IgG antibody (Jackson). Ankyrin-G staining was visualized with a Texas red-conjugated anti-chicken IgY antibody (Jackson). Arrows indicate colocalized sodium channel and ankyrin-G clusters. Scale bar, $25 \mu\text{m}$.

light, diffuse axonal immunoreactivity was present. At P10, when nearly all astrocytes have developed, only a few sodium-channel clusters had formed. By P16, when about half of the oligodendrocytes have developed, many axonal sodium-channel clusters were present. These results show that oligodendrocytes and sodium-channel clusters develop concurrently in rat optic nerve.

To examine more directly whether oligodendrocytes are necessary for sodium-channel clustering *in vivo*, we compared the amount of clustering in optic-nerve cryosections from wild-type and *myelin-deficient* (*md*) rats¹⁷ that are deficient in oligodendrocytes but not astrocytes or neurons^{18,19} (Fig. 4a). At both time points examined, the amount of sodium-channel clustering was significantly reduced concomitant with the reduction in oligodendrocyte number (Fig. 4b–f). Previous studies have demonstrated that the absolute number of sodium channels in these rats does not differ from wild-type animals²⁰, suggesting that the deficiency of oligodendrocytes specifically impairs sodium-channel clustering and not synthesis. Together, these findings show that the formation of axonal sodium-channel clusters *in vivo* depends on the presence of oligodendrocytes.

The ability to culture purified RGCs in the presence or absence of glial cells⁷, together with the development of a highly specific sodium-channel antibody³, has allowed us to investigate the cell–cell interactions that induce sodium-channel clustering along CNS axons. Our findings show that retinal ganglion cells are intrinsically unable to form sodium-channel clusters in the absence of glial cells, even though they express sodium channels, are excitable, form dendrites and axons, and target sodium channels appropriately to axons⁷. Clustering of sodium channels along RGC axons is induced by a soluble protein signal produced by oligodendrocytes. Although previous studies have demonstrated a close spatial and temporal correlation between astrocyte–axon contact and the appearance of a high density of intramembrane particles^{5,6}, our observations demonstrate that oligodendrocytes are the source of sodium-channel clustering activity both *in vitro* and *in vivo*. Our data do not exclude the possibility, however, that glial contact enhances the recruitment of sodium channels to the nodal membrane from an intra-axonal storage pool. As glia also induced ankyrin-G clustering in a pattern identical to that of sodium-channel clustering, it is likely that cytoskeletal interactions play an important role in the induction and maintenance of the sodium-channel clusters¹⁴.

These findings have several implications. First, many previous

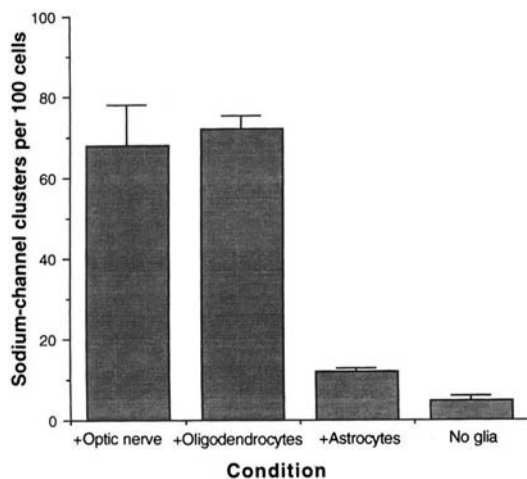


Figure 2 Comparison of sodium-channel cluster-inducing activity from astrocytes and oligodendrocytes *in vitro*. Values are the mean number of clusters per 100 cells from all coverslips counted ($\pm$ s.e.m.). The percentage of neurons with axons (identified by a stained initial segment) having at least one cluster was 48 ± 1 (plus mixed optic nerve), 35 ± 1 (plus oligodendrocytes), 28 ± 1 (plus astrocytes), and 12 ± 1 (no glial cells). The estimated average number of clusters per positive cell was 3 (plus mixed optic nerve), 5 (plus oligodendrocytes) and 1 (plus astrocytes and no glial cells).

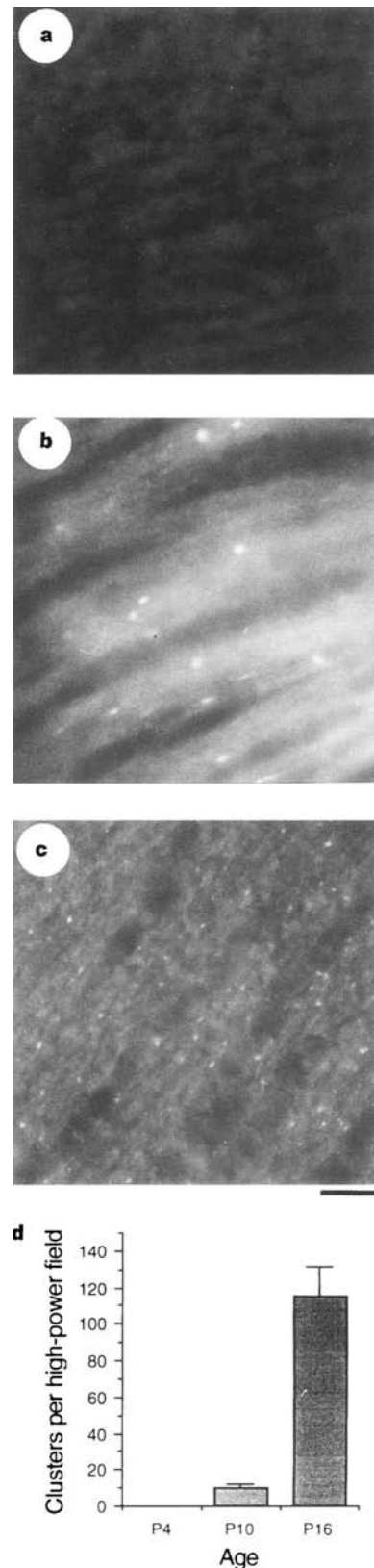


Figure 3 The time course of development of sodium-channel clusters in rat optic nerve, as shown by immunostaining of P4 (a), P10 (b) and P16 (c) optic-nerve cryosections. Quantification of clustering is shown in d. Scale bar, 25 μ m. The difference in sizes between clusters at different ages is also seen *in vivo*^{5,6}. Similar patterns of immunoreactivity were seen with ankyrin-G.

studies have suggested that contact with glial cells determines nodal and internodal domains^{3,5,6,21}. In contrast, our findings strongly suggest that nodal and internodal domains are initially specified by the axon, with glial contact occurring subsequently. A potential molecular basis for this possibility is suggested by findings that cell adhesion molecules are restricted to nodal regions of the axon, including neurofascin²², an L1 homologue, and the $\beta 2$ subunit of the sodium channel, a contactin homologue²³. Both of these proteins bind to known astrocyte surface molecules. Second, our finding that nodal domains, once induced, persist in the absence of glia raises the possibility that the conduction deficit caused by multiple sclerosis, in which oligodendrocytes die for unknown reason, may not only be caused by demyelination but because sodium channels remain clustered after demyelination^{24,25}. Finally, together with previous observations, our findings show that oligodendrocytes are specialized to increase conduction velocity by at least three mechanisms: they provide myelin insulation; they promote the radial growth of axons²⁶; and they induce clustering of sodium channels, thereby enabling saltatory conduction. □

Methods

Characterization of Na⁺ channel antibody. The antibody was raised in rabbit to a conserved segment of the intracellular III–IV loop and affinity purified³. In

a western blot on P16 optic-nerve extract, the antibody detected a band at 260K, the expected M_r of the sodium-channel α -subunit²⁷. This band was eliminated by preabsorption of the antibody with an excess of the immunizing peptide (data not shown). In addition, in cryosections of P16 optic nerves, the antibody lightly labelled axons and strongly labelled axonal clusters of the approximate dimensions of nodes; glial cell bodies were not labelled (Fig. 3). All cryosection immunoreactivity was eliminated by preabsorption with the immunizing peptide (data not shown).

Immunocytochemistry. Cryosections were prepared from rats that were anaesthetized with ether and perfused with 4% paraformaldehyde. The optic nerves were incubated in 4% paraformaldehyde, transferred to 30% sucrose in PBS until equilibrated, frozen with OCT compound (Miles) and cut into 10- μ m longitudinal sections with a cryostat. The sections were collected onto gelatinized glass slides. Sections and cultures were immunostained³, mounted in Vectashield (Vector), and examined using a Nikon Microphot-FXA fluorescence microscope. Primary antibodies used were mouse anti-MAP2 (Sigma), rabbit anti-sodium channel³, and chicken anti-ankyrin-G.

Preparation of cultures. RGCs from P8 S/D rats (Simonsen, CA) were purified to greater than 99.5% purity by immunopanning and cultured in serum-free medium containing BDNF, CNTF, insulin and forskolin, as previously described^{7,28}. Conditioning cultures of P8 optic-nerve glial cells were prepared⁷. Oligodendrocyte-conditioning layers were prepared by purifying oligodendrocytes to greater than 99.9% purity by immunopanning from

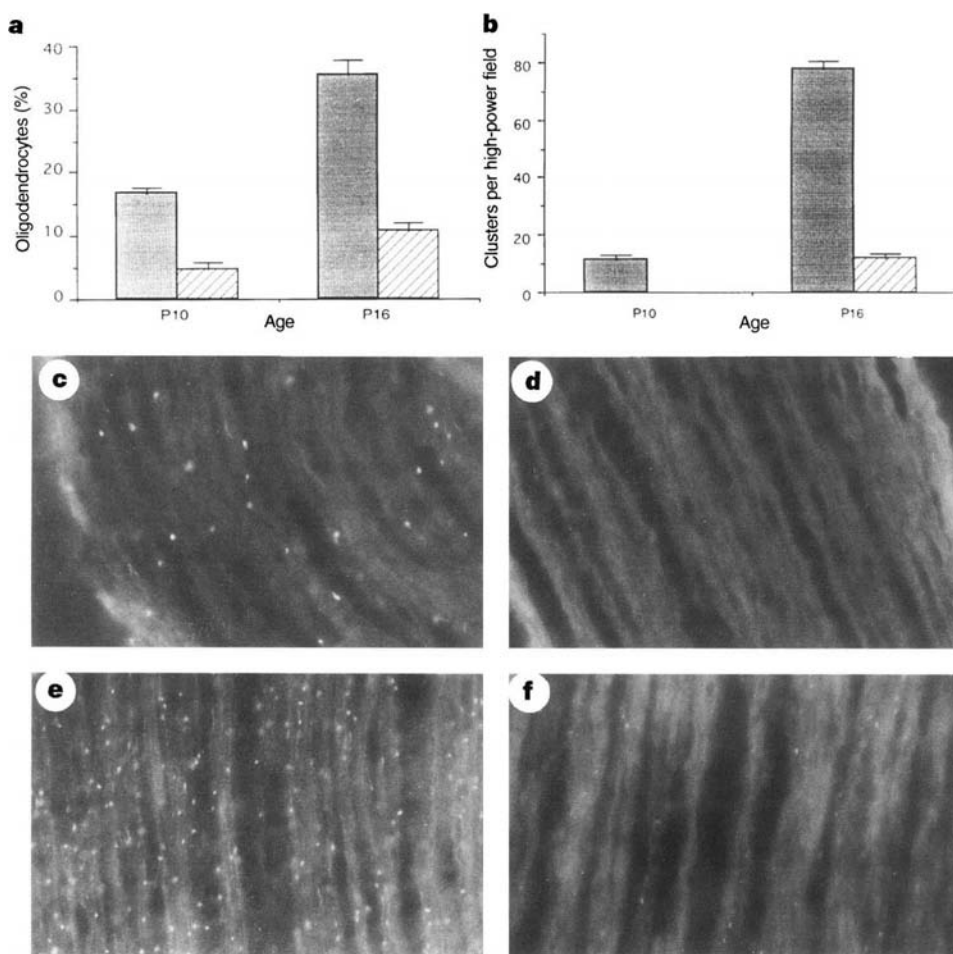


Figure 4 **a**, Percentage of cells that are oligodendrocytes in wild-type (solid bars) versus *myelin-deficient* (hatched bars) rats. Cryosections were stained with CC-1 (ref. 30), an oligodendrocyte marker, and bisbenzamide, a nuclear stain. The percentage of CC-1-positive cells was then determined. **b**, Number of sodium-channel clusters seen in wild-type (solid bars) versus *myelin-deficient* (hatched

bars) rats. The number of clusters per high power ($\times 60$) field was counted. **c–f**, Immunostaining of P10 (**c**, **d**) and P16 (**e**, **f**) cryosections with sodium-channel antibody, wild-type (**c**, **e**) versus mutant (**d**, **f**); $N = 4$ rats per age and condition. Values are mean $\pm$ s.e.m. Scale bar, 25 μ m.

the optic nerves of P7 S/D rats¹⁵. Approximately 50,000 cells were plated per well in PDL-coated 24-well plates. The cells were cultured in serum-free medium containing NT3, CNTF, insulin and forskolin¹⁵. After 6 days, the medium was replaced with RGC culture medium. Astrocyte-conditioning layers were prepared by purifying astrocytes to greater than 99.9% purity by immunopanning from the optic nerves of P1 S/D rats²⁹. Approximately 30,000 cells were plated per well in 24-well plates in serum-free medium with BDNF, CNTF, insulin, forskolin and bFGF (Peprotech) to promote the survival and proliferation of the astrocytes. After 7 days, cells were washed and replaced with the medium described for RGCs. Medium was conditioned by the oligodendrocytes or astrocytes for 2 days before the addition of coverslips with cultured RGCs; the RGC coverslips were separated from the glial-conditioning layer by glass-chip pedestals.

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Suppression of signalling through transcription factor NF-AT by interactions between calcineurin and Bcl-2

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It is not known how the protein Bcl-2 inhibits cell death induced by calcium signalling and growth-factor withdrawal^{1–3}. Here we report that Bcl-2 forms a tight complex with calcineurin, resulting in the targeting of calcineurin to Bcl-2 sites on cytoplasmic membranes, and show that this interaction is dependent on the BH4 domain of Bcl-2. Calcineurin bound to Bcl-2 is an active phosphatase but is unable to promote the nuclear translocation of NF-AT, a transcription-factor required for induction of interleukin-2 expression, suggesting a mechanism by which Bcl-2 suppresses NF-AT activity⁴. We also show that Bax, a pro-apoptotic member of the Bcl-2 family, interferes with interactions between calcineurin and Bcl-2. We propose that the ability of Bcl-2 to block NF-AT signalling is due to the sequestering of active calcineurin to the same domain of Bcl-2 which associates with Rad-1 (ref. 5), and that calcineurin may act in Bcl-2-regulated functions.

Bcl-2 suppresses NF-AT signalling in T cells of Bcl-2 transgenic mice, and blocks cell death resulting from calcineurin overexpression^{4,6}. To examine the possibility that Bcl-2 interacts with calcineurin, myc-epitope-tagged Bcl-2 was coexpressed in baby hamster kidney (BHK) cells with either calcineurin (CnA), Bax or control proteins, and immunoprecipitated with anti-myc antibodies⁷ (Fig. 1a). In addition to Bax, both CnA and ΔCnA, a constitutively active mutant of calcineurin, coprecipitate with Bcl-2 (Fig. 1a). Interactions between calcineurin and Bcl-2 also were detected in Jurkat cells stably expressing Bcl-2, and in the SU-DHL-4 B-cell lymphoma cell line⁸, which expresses high levels of Bcl-2 (Fig. 1b, c). Because calcineurin is found in the cytoplasm in a diffuse cytoplasmic distribution⁶, whereas Bcl-2 is found on endoplasmic reticulum and mitochondrial membranes^{9–11}, their interaction in the cell should alter the distribution of one or both of these proteins. Such an interaction-dependent redistribution in the cell can be demonstrated using the known dimerization properties of Bcl-2 and Bax^{12,13}. Expression of myc-Bcl-2 alone shows the expected cytoplasmic membrane pattern (Fig. 1d, right), as does haemagglutinin (HA)–Bax expressed at very low levels (not shown). At higher levels of expression, HA–Bax shows a diffuse cytoplasmic and nuclear distribution (Fig. 1d, middle). However, coexpression of Bcl-2 and Bax results in a dramatic relocation of Bax to the Bcl-2 staining cytoplasmic membranes (Fig. 1d, left), indicating that Bax, despite its transmembrane domain, requires Bcl-2 to associate with cytoplasmic membranes. Similarly, calcineurin is relocated to cytoplasmic membranes in the presence of Bcl-2 (Fig. 1e). Control proteins, including cyclophilin A, NF-AT4, or the catalytic A subunit of calcineurin (CnA), in the absence of its regulatory B subunit (CnB) (not shown), show no redistribution as a consequence of Bcl-2 coexpression.

To determine which domains of Bcl-2 are required for the observed calcineurin interaction, calcineurin was coexpressed in cells with Bcl-2 mutants lacking the transmembrane domain (ΔTM–Bcl-2)^{14,15}, residues 1–81 of the amino-terminal domain

(Δ N-Bcl-2), or residues 82–239 of the carboxy terminus (Δ C-Bcl-2) (Fig. 2a). Calcineurin coprecipitates with Δ TM-Bcl-2 and Δ C-Bcl-2, but not with Δ N-Bcl-2 (Fig. 2b). To localize more precisely the site within the N terminus of Bcl-2 responsible for calcineurin binding, green fluorescent protein (GFP)¹⁶ was fused to different portions of the Bcl-2 N terminus, synthesized by *in vitro* translation, and assayed for binding to calcineurin (Fig. 2c, d). The first 20

amino acids of Bcl-2 are sufficient for the coprecipitation of calcineurin. This region corresponds to most of an α -helical domain in the anti-apoptotic Bcl-2 homologue Bcl-x_L¹⁷ (α -helix 1, residues 4–19)¹⁸; the pro-apoptotic Bcl-2 family members, including Bax and Bak, lack this domain (Fig. 2e).

The *in vitro* translation data also show that Δ TM-Bcl-2 interacts strongly with the catalytic subunit (Δ CnA) when complexed with the regulatory subunit (CnB). This dependence on CnB is consistent with the observation that the regulatory B subunit of calcineurin plays an important role in the structure and activity of CnA¹⁹.

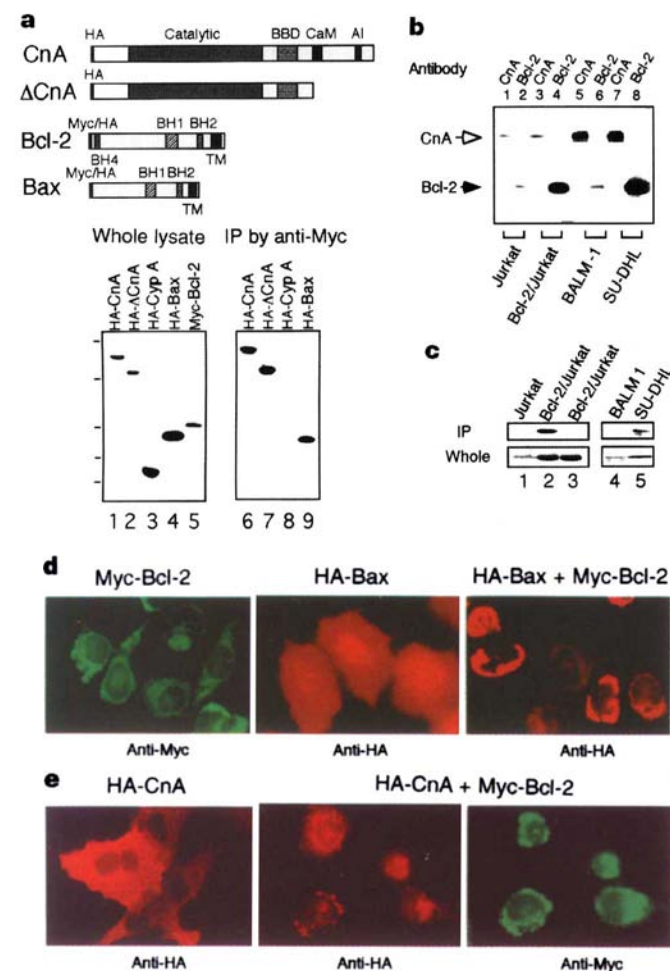


Figure 1 Intracellular interactions between calcineurin and Bcl-2. **a**, Top, schematic structures of calcineurin A (CnA), constitutively active calcineurin A (Δ CnA), Bcl-2 and Bax, and their expression and interaction on transfection of BHK cells. Bottom, immunoblots of whole-cell lysates revealing the expression of HA-CnA, HA- Δ CnA, HA-cyclophilin A (CypA), myc-Bcl-2, and HA-Bax (left), and Bcl-2 immunoprecipitates (IP) with anti-myc tag antibodies revealing the coprecipitation of CnA, CnA and Bax, but not of CypA (right). **b**, Analysis of calcineurin and Bcl-2 expression in various T- and B-cell lines using anti-calcineurin and anti-Bcl-2 antibodies on immunoblots of whole-cell lysates. T-cells include the human Jurkat T-cell line and a Jurkat cell line stably expressing Bcl-2. B cells include the BALM-1 B-cell leukaemia cell line, which expresses low levels of Bcl-2, and the SU-DHL-4 B-cell lymphoma line, expressing high levels of Bcl-2. **c**, Detection of Bcl-2 in calcineurin immunoprecipitates from whole-cell lysates of Bcl-2-Jurkat cells (lane 2) and SU-DHL-4 cells (lane 5), but not in Jurkat (lane 1), BALM-1 cells (lane 4) or in Bcl-2-Jurkat cells (lane 3) immunoprecipitated with control, anti-GFP monoclonal antibody (MIC, Boston). **d**, Subcellular localization of myc-Bcl-2 (left) on cytoplasmic membranes, and HA-Bax (middle) to cytoplasm and nucleus. On coexpression of Bcl-2 and Bax, Bax assumes a cytoplasmic membrane distribution identical to that of Bcl-2 (right). **e**, HA-CnA assumes a diffuse cytoplasmic distribution when expressed in BHK cells (left). HA-CnA is translocated to cytoplasmic membranes when coexpressed with myc-Bcl-2 (middle and right).

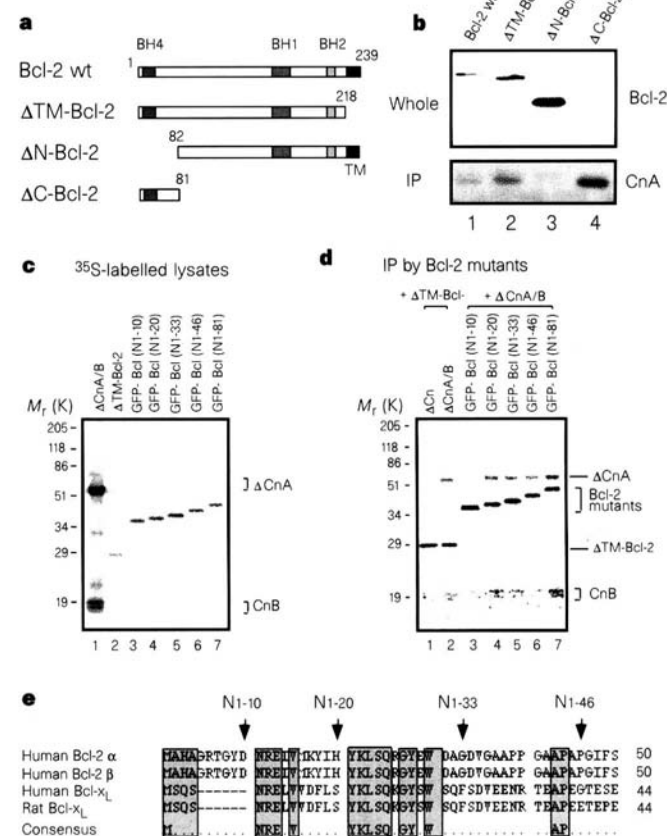


Figure 2 Calcineurin interacts with the BH4 domain of Bcl-2. **a**, Schematic structures of myc-Bcl-2 mutants coexpressed with HA-calcineurin in BHK cells. Δ TM-Bcl-2 lacks the transmembrane domain (deletion of amino acids 218–239), Δ N-Bcl-2 lacks the first 81 amino acids, and Δ C-Bcl-2 lacks the C-terminal 158 amino acids of Bcl-2. **b**, Calcineurin coprecipitates with wild-type Bcl-2. Δ TM-Bcl-2 and Δ C-Bcl-2 in whole-cell lysates of BHK cells coexpressing the indicated proteins, but fails to interact with Δ N-Bcl-2. **c**, ³⁵S-labelled *in vitro* translation of calcineurin subunits (Δ CnA at 44K; CnB at 19K), and of Δ TM-Bcl-2 (24K) and GFP-Bcl-2 fusion proteins in reticulocyte extracts. **d**, Immunoprecipitation of Bcl-2 mutants and fusion proteins following coexpression with calcineurin subunits. The coprecipitation of calcineurin with Δ TM-Bcl-2 depends on the expression of the calcineurin B subunit, which is essential for the activity and structure of the catalytic subunit of calcineurin (lanes 1 and 2). CnA/CnB coprecipitates with GFP-Bcl-2 fusion proteins that contain at least the first 20 amino acids of Bcl-2 (ref. 23) (lanes 3–7). **e**, Alignment of the N-terminal sequences of Bcl-2 and Bcl-x_L showing the degree of conservation in the region of Bcl-2 (amino acids 1–20) required for interaction with calcineurin.

The functional consequences of interactions between Bcl-2 and calcineurin for NF-AT4 nuclear import were tested in BHK cells. In the absence of Bcl-2, NF-AT4 is rapidly imported to the nucleus in response to calcium ionophore in a manner dependent on calcineurin activity (Fig. 3a)¹⁹. However, Bcl-2 blocks ionophore-activated NF-AT4 nuclear import (Fig. 3b, c) in a dose-dependent manner. In addition, Bcl-2 expression prevents the calcineurin-dependent dephosphorylation of NF-AT4 (Fig. 3c). The suppression of NF-AT4 nuclear translocation by Bcl-2 is relieved by the coexpression of Bax (Fig. 3a, b). Transcriptional activation of an NF-AT reporter gene was compared in Jurkat cell lines expressing low and high levels of Bcl-2. In normal Jurkat cells, an NF-AT reporter gene shows high expression, owing to NF-AT mobilization in the presence of calcium ionophore and the phorbol ester TPA, whereas the Jurkat cell line overexpressing Bcl-2 shows a low level of NF-AT reporter activation (Fig. 3d). In contrast, Bcl-2 has no

obvious effect on the activation of an NK- κ B reporter gene (Fig. 3d).

The mechanism by which Bcl-2 suppresses the NF-AT pathway could involve sequestering active calcineurin at cytoplasmic membranes, or the inhibition of calcineurin activity through the binding process itself. To distinguish between these possibilities, we investigated whether Δ TM-Bcl-2, which binds but does not sequester calcineurin, can still disrupt signalling through NF-AT. Unlike Bcl-2, Δ TM-Bcl-2 is distributed diffusely in the cytoplasm and nucleoplasm (Fig. 4a). Calcium ionophore stimulation of cells coexpressing Δ TM-Bcl-2 and NF-AT4 showed a rapid translocation of NF-AT4 to the nucleus with kinetics similar to that observed in cells transfected with NF-AT4 alone (Fig. 4a). Further, Δ TM-Bcl-2 expression does not interfere with the calcineurin-dependent dephosphorylation of NF-AT4 during its transport (Fig. 4b, lane 7). We also found that the electrophoretic migration of Δ TM-Bcl-2, unlike Bcl-2, is altered in a calcium- and calcineurin-dependent manner, suggesting that calcineurin can dephosphorylate Δ TM-Bcl-2 directly (Fig. 4b, lanes 6–8). Further analysis will be required to understand the modifications underlying this mobility shift of Δ TM-Bcl-2 and whether wild-type

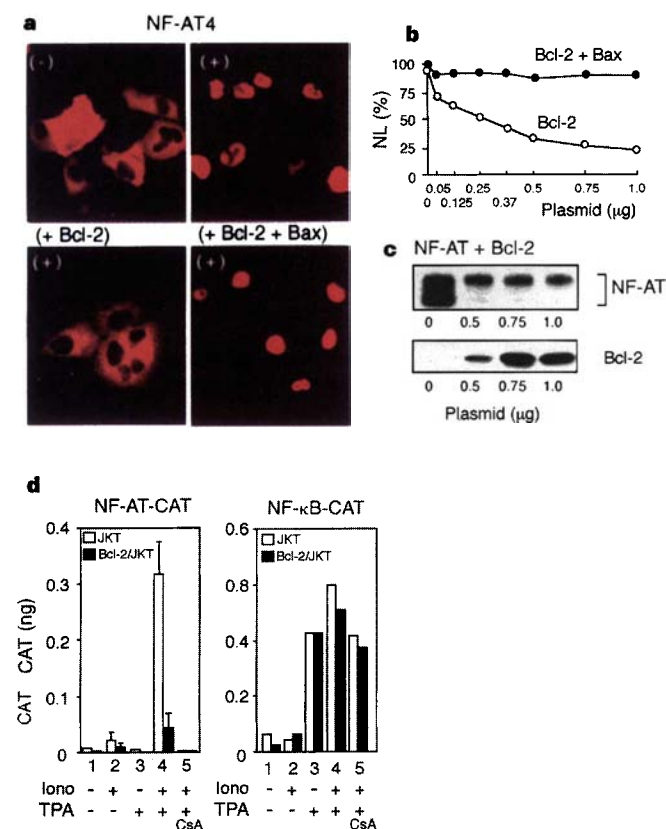


Figure 3 Bcl-2 suppresses calcineurin-dependent NF-AT signalling. **a**, Immunolocalization of HA-NF-AT4 expression in transfected BHK cells in the absence (top left) and presence (top right) of calcium ionophore. Calcium ionophore results in the nuclear translocation of NF-AT4 over 30 min. Coexpression of HA-NF-AT4 and myc-Bcl-2 does not alter the localization of NF-AT4 in unstimulated cells, but suppresses the ionophore-activated nuclear import of NF-AT4 (bottom left). This suppression of NF-AT4 nuclear import is relieved by the expression of myc-Bax (bottom right). **b**, Dose-dependent suppression of NF-AT4 nuclear import by Bcl-2 is reversed by the transfection of Bax at equimolar ratios (NL, nuclear localization). **c**, Top, suppression of calcineurin-dependent dephosphorylation of NF-AT4 by Bcl-2. HA-NF-AT4 was expressed with increasing levels of myc-Bcl-2 plasmid in BHK cells that were treated with calcium ionophore 30 min before lysis and western analysis. Bottom, immunoblot analysis of same lysates with anti-myc antibody. **d**, Bcl-2 selectively blocks NF-AT-dependent transcriptional activation. Jurkat and Bcl-2/Jurkat cells were transfected with either NF-AT or NF- κ B reporter genes and analysed for transcriptional activation after exposure to calcium ionophore and TPA for 16 h. NF- κ B-CAT expression is relatively unaffected by the expression of Bcl, but NF-AT-CAT expression is markedly suppressed in the Jurkat line overexpressing Bcl-2.

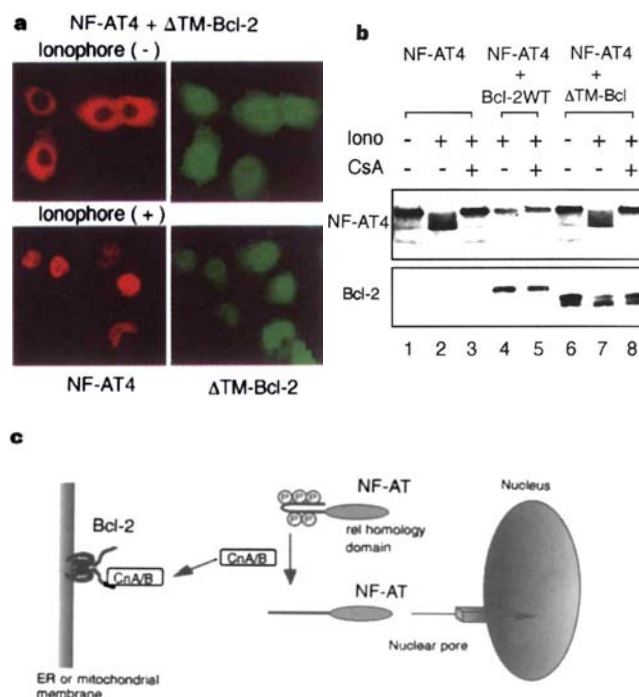


Figure 4 Δ TM-Bcl-2 does not suppress signalling through NF-AT. **a**, Cytoplasmic localization of HA-NF-AT4 (top right) is unstimulated BHK cells coexpressing myc- Δ TM-Bcl-2 (top left). Nuclear localization of NF-AT4 after 30 min calcium ionophore treatment in BHK cells coexpressing Δ TM-Bcl-2 (bottom panels). **b**, Effect of Δ TM-Bcl-2 on NF-AT4 dephosphorylation in response to calcium ionophore. Phosphorylated NF-AT in unstimulated BHK cells (lane 1) is converted to high-mobility form in the presence of calcium ionophore (lane 2). Coexpression of Bcl-2 blocks the calcium ionophore-induced mobility shift of NF-AT4 (lane 4); Δ TM-Bcl-2 expression does not affect the mobility shift (lanes 6–8). Cyclosporin A (500 nM) blocks the calcium-dependent mobility shift of NF-AT4 in the presence of Δ TM-Bcl-2 (lane 8). **c**, Model for the inhibition of NF-AT₄ signalling by Bcl-2. Calcineurin induces the nuclear import of NF-AT₄ by binding and dephosphorylation of N-terminal serine residues. In the presence of excess Bcl-2, calcineurin is sequestered at the BH₄ domain and is unavailable for complexing with NF-AT₄ and co-transport to the nucleus.

Bcl-2 undergoes similar modifications in response to calcineurin activity. Overall, these data support the notion that the mislocalization of calcineurin by Bcl-2 renders it unavailable for NF-AT dephosphorylation, and that calcineurin in a complex with Bcl-2 retains its catalytic activity.

Our data demonstrate interactions between Bcl-2 and calcineurin, and thereby provide a molecular explanation for the specific disruption of NF-AT signalling by Bcl-2 (ref. 4). The heterodimerization of Bcl-2 with Bax abolishes the sequestering of calcineurin by Bcl-2, and restores calcineurin-dependent NF-AT4 signalling. This finding suggests that interactions between calcineurin and Bcl-2 can be regulated by other, pro-apoptotic members of the Bcl-2 family. Whether these interactions regulate the anti-apoptotic function of Bcl-2 in T cells remains uncertain. Although NF-AT signalling is considered essential for activation-induced cell death in T cells^{4,6,20}, it is not known whether interactions between Bcl-2 and calcineurin disrupt this process or contribute to lymphoproliferation²¹ following Bcl-2 overexpression. At least in BHK cells, Bcl-2 lacking the BH4 domain acts as a pro-apoptotic molecule (F.S. and E.K., unpublished observations). Similar pro-apoptotic Bcl-2 variants have been generated by introducing point mutations in the BH4 domain²², although it is not known whether these mutants interact with calcineurin or Raf-1. Such a simple scheme is inadequate to explain the diverse effects of Bcl-2 on cell-cycle control in the G1 phase and its ability to activate Raf-1 independent of growth-factor stimulation^{1,3,5}. Alternatively, the observed ability of Bcl-2 to disrupt NF-AT signalling may simply be a by-product of its strong interaction with calcineurin, which in turn suggests that calcineurin is involved in regulating Bcl-2 and related proteins. In support of this notion, we show that calcineurin binds the BH4 domain of Bcl-2, a region only found in Bcl-2, Bcl-X_L, and other anti-apoptotic members of the Bcl-2 family¹⁸. Moreover, we show that Bax dimerization with Bcl-2 prevents the binding of calcineurin. Finally, we demonstrate that the calcineurin, in a complex with Δ TM-Bcl-2, is still active and dephosphorylates NF-AT4 in a manner dependent on calcineurin activity (Fig. 4b). These observations indicate that calcineurin retains catalytic activity when in a complex with Bcl-2. The idea that an active calcineurin binds the BH4 domain of Bcl-2 is interesting because independent analyses have revealed specific interactions between the protein kinase Raf-1 and the BH4 domain of Bcl-2, which result in the activation of Raf-1 (ref. 5). Thus the convergence of both a protein kinase, Raf-1, and a phosphatase, calcineurin, onto the same domain of Bcl-2 suggests that there are functional interactions between these proteins in the mechanism of Bcl-2-regulated cell death. □

Methods

Plasmid constructs. Mammalian expression vectors for CnA, CnB, Δ CnA and NF-AT4 and BHK transfection protocols have been described previously⁹. The cDNA encoding NG-AT4a was provided by T. Hoey²⁰. The cDNAs encoding human Bcl-2 and mouse Bax were gifts from S. Korsmeyer. The pcDNA3 vectors (Stratagene) expressing *myc*- and *HA*-tagged human Bcl-2 and murine Bax were constructed using the polymerase chain reaction (PCR).

NF-AT4 nuclear import assays. Transfection of plasmids was performed as previously described⁶. BHK cells that were cotransfected with *HA*-NF-AT4 and *myc*-Bcl-2 or *myc*- Δ TM-Bcl-2 were prepared for NF-AT4 immunolocalization after treatment with or without calcium ionophore (1 μ M A₂₃₁₈₇) for 30 min. NF-AT4 nuclear import was detected using the anti-*HA* polyclonal antibody and a Cy3-labelled goat anti-rabbit antibody. For NF-AT4 mobility shifts, whole-cell lysates of BHK cells transfected with the indicated expression plasmids were fractionated on 8.5% SDS-polyacrylamide gels and transferred to Immobilon (Millipore) membranes. Membranes were probed with the anti-*HA* polyclonal antibodies (MIC, Boston) for detection of *HA*-NF-AT4 or anti-*myc* polyclonal antibody (MIC, Boston) for *myc*-Bcl-2 and revealed by horseradish peroxidase-conjugated secondary antibodies and chemiluminescence (ECL, Amersham).

Immunoprecipitations. Transfected BHK cells on coverslips were washed with

ice-cold PBS twice and lysed in 300 μ l of lysis buffer A (20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1 mM EGTA, 1 mM MgCl₂, 0.2% Triton X-100, 1 mM DTT, 10% glycerol, 1 mM PMSF, 1 μ g ml⁻¹ Leupeptin and 1 μ g ml⁻¹ Pepstatin), transferred to a microfuge tube, and centrifuged at 10,000g for 10 min at 4°C. Supernatants were transferred to new tubes and preabsorbed by 30 μ l of Sepharose 4B beads for 20 min. After centrifugation, 30 μ l of protein G-Sepharose (Pharmacia, 1:1 slurry, Sigma) conjugated with the anti-*myc* epitope (9E10) monoclonal antibody (Babco) or the anti-Bcl-2 monoclonal antibody (Dakopatts, Denmark) was added. Tubes were rotated at 4°C for 1 h. The beads were pelleted by centrifugation and washed three times by 500 μ l of lysis buffer. For the preparation of lysates from lymphoma leukaemia cell lines (Hayashibara Institute, Okayama, Japan) or Jurkat cell lines, 5 $\times$ 10⁵ cells were lysed with lysis buffer A. After centrifugation, each supernatant was mixed with protein G-Sepharose beads preincubated both with a mouse anti-calcineurin A monoclonal antibody (Vj6) and mouse anti-calcineurin B monoclonal antibodies (Val) (Vj6 and Val provided by H. Matsui) overnight at 4°C. After washing the beads five times, bound proteins were fractionated in 7.5% or 12.5% SDS-PAGE by electrophoresis and transferred to PVDF membranes (Immobilon, Millipore). Bcl-2 bound to calcineurin was detected by anti-Bcl-2 antibody. Anti-GFP monoclonal antibody (MBL, Japan) was used as a control. ³⁵S-labelled *in vitro* transcription/translation (Promega) reactions and NF- κ B- and NF-AT-CAT assays were performed as previously described¹⁹. Jurkat cells stably expressing Bcl-2 were constructed by electroporation of pcDNA3-Bcl-2 and selection by G418 (GIBCO-BRL, 200 μ g ml⁻¹). The positive clones were selected by limiting dilution.

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Functional interaction between DNA-PK and c-Abl in response to DNA damage

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How DNA damage is converted into intracellular signals that can control cell behaviour is unknown. The c-Abl protein tyrosine kinase is activated by ionizing radiation and certain other DNA-damaging agents^{1–5}, whereas the DNA-dependent protein kinase (DNA-PK), consisting of a serine/threonine kinase and Ku DNA-binding subunits, requires DNA double-strand breaks or other DNA lesions for activation^{6–8}. Here we demonstrate that c-Abl interacts constitutively with DNA-PK. Ionizing radiation stimulates binding of c-Abl to DNA-PK and induces an association of c-Abl with Ku antigen. We show that DNA-PK phosphorylates and activates c-Abl *in vitro*. Cells deficient in DNA-PK are defective in c-Abl activation induced by ionizing radiation. In a potential feedback mechanism, c-Abl phosphorylates DNA-PK, but not Ku, *in vitro*. Phosphorylation of DNA-PK by c-Abl inhibits the ability of DNA-PK to form a complex with DNA. We also show that treatment of cells with ionizing radiation results in phosphorylation of DNA-PK that is dependent on c-Abl. Our results support the hypothesis that there are functional interactions between c-Abl and DNA-PK in the response to DNA damage.

To identify proteins that associate with nuclear c-Abl, we analysed anti-c-Abl immunoprecipitates by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and silver staining. The detection of a coprecipitated protein of relative molecular mass (M_r) of about 450K was confirmed to be DNA-PK by reactivity with an anti-DNA-PK antibody (Fig. 1a). DNA-PK is involved in DNA double-strand break (DSB) repair and V(D)J recombination^{9–12}. Because ionizing radiation (IR) induces DNA DSBs and activates c-Abl¹, we investigated whether IR affects the interaction between c-Abl and DNA-PK. Exposure to radiation led to an increase in the association of c-Abl and DNA-PK (Fig. 1a). When anti-DNA-PK immunoprecipitates were analysed by immunoblotting with anti-c-Abl in the reciprocal experiment, the results confirmed IR-induced binding of c-Abl and DNA-PK (Fig. 1b). The association between c-Abl and DNA-PK was further studied by incubating U-937 cell extracts with glutathione-S-transferase (GST) fusion proteins prepared from full-length c-Abl. Adsorbates obtained with GST-c-Abl, and not with GST, demonstrated binding with DNA-PK (Fig. 1c). Adsorbates obtained with GST-Abl SH3, but not with GST fusion proteins derived from the amino- or carboxy-terminal SH3 domains of Grb2, also revealed binding of DNA-PK (Fig. 1c). The c-Abl SH3 domain binds to proline-rich sequences with the consensus XPXXPX^{13,14}. The identification of at least six potential sequences for c-Abl SH3 binding in DNA-PK suggested that there is a direct interaction between these two proteins. Indeed, the results of binding assays with purified DNA-PK demonstrated a direct interaction with c-Abl (data not shown). The Ku antigen, a DNA end-binding protein and transcription factor, activates DNA-PK^{8,15,16}. There was little if any detectable interaction of c-Abl and Ku in U-937 cells (Fig. 1d). However, IR treatment resulted in the formation of complexes containing c-Abl

and Ku (Fig. 1d). The effects of DNA damage on the interaction between c-Abl and DNA-PK are not limited to IR treatment as similar findings were obtained with the DNA alkylating agent methyl methanesulphonate (MMS) (Fig. 1e).

To assess whether the interaction between c-Abl and DNA-PK affects c-Abl, we incubated purified heat-inactivated c-Abl with purified DNA-PK and Ku in the presence of sonicated DNA. The results demonstrate phosphorylation of c-Abl on serine as confirmed by phosphoamino-acid analysis (Fig. 2a and data not shown). No phosphorylation was observed in the absence of sonicated DNA (Fig. 2a). The effect of DNA-PK on c-Abl activity was determined in similar assays with kinase-active c-Abl and the c-Abl substrate GST-Crk(120–225). The presence of activated DNA-PK (with sonicated DNA) increased c-Abl-dependent phosphorylation of GST-Crk (Fig. 2b). Immunoblotting with anti-P-Tyr confirmed tyrosine phosphorylation of GST-Crk (data not shown).

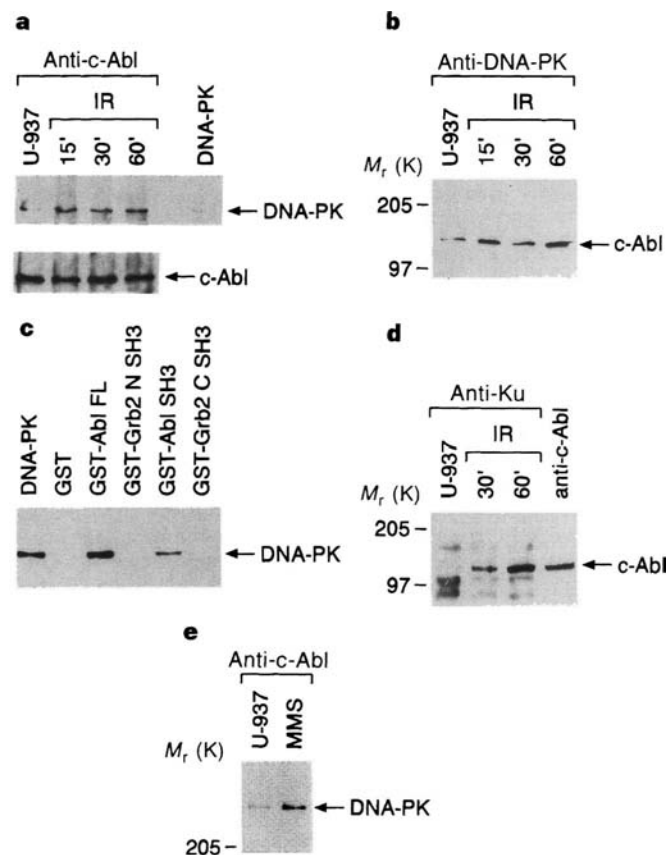


Figure 1 Binding of c-Abl and DNA-PK. **a**, U-937 cells were treated with 20 Gy ionizing radiation (IR) and collected at the indicated times (in min). Total cell lysates were subjected to immunoprecipitation with anti-c-Abl. Purified DNA-PK was used as a positive control. Immunoprecipitates were then analysed by immunoblotting with anti-DNA-PK (top panel). Immunoprecipitates were also analysed by immunoblotting with anti-c-Abl (bottom panel). **b**, Lysates from control and IR-treated cells were immunoprecipitated with anti-DNA-PK and the precipitates were analysed by immunoblotting with anti-c-Abl. **c**, IR-treated cell lysates were incubated with GST, GST-c-Abl (full-length; FL), GST-Grb2 N.S.H3 (Santa Cruz Biotechnology, Santa Cruz, CA), GST-Abl-SH3 or GST-Grb2 C.S.H3 (Santa Cruz) fusion proteins. The adsorbates were analysed by immunoblotting with anti-DNA-PK. Purified DNA-PK was used as a positive control (lane 1). **d**, Lysates were subjected to immunoprecipitation with anti-Ku. Lysate from IR-treated cells was also immunoprecipitated with anti-c-Abl as a positive control. The protein precipitates were analysed by immunoblotting with anti-c-Abl. **e**, Cells were exposed to 1 mM MMS for 30 min. Lysates were subjected to immunoprecipitation with anti-c-Abl and the precipitates were analysed by immunoblotting with anti-DNA-PK.

These findings indicated that phosphorylation of c-Abl on serine by DNA-PK stimulates c-Abl activity *in vitro*. To determine whether DNA-PK contributes to the activation of c-Abl in irradiated cells, we compared the effects of IR on c-Abl activity in DNA-PK^{-/-} and DNA-PK^{+/+} cells. Whereas c-Abl activity was stimulated about 1.5-fold in IR-treated DNA-PK^{-/-} cells, irradiation of DNA-PK^{+/+} cells exhibited a 2.9-fold stimulation (Fig. 2c). These results indicated that DNA-PK contributes at least in part to IR-induced c-Abl activation.

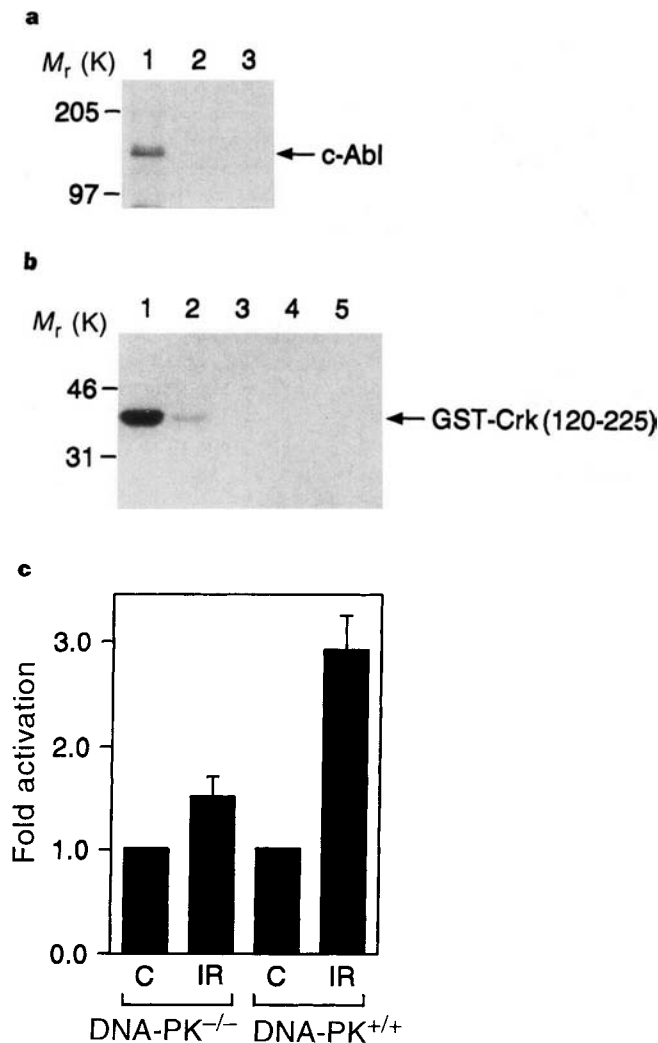


Figure 2 DNA-PK phosphorylates and activates c-Abl. **a**, Purified DNA-PK/Ku was incubated with purified heat-inactivated (HI) c-Abl in the presence and absence of sonicated DNA (lane 1, DNA-PK/Ku + HI c-Abl + sonicated DNA; lane 2, HI c-Abl alone; and lane 3, DNA-PK/Ku + HI c-Abl without sonicated DNA). **b**, c-Abl was activated *in vitro* by incubation with purified DNA-PK/Ku. Phosphorylated proteins were analysed by autoradiography (lane 1, DNA-PK/Ku + c-Abl + sonicated DNA + GST-Crk; lane 2, DNA-PK/Ku + c-Abl + GST-Crk; lane 3, DNA-PK/Ku + HI c-Abl + sonicated DNA + GST-Crk; lane 4, DNA-PK/Ku + GST-Crk; and lane 5, GST-Crk alone). **c**, Mouse DNA-PK^{-/-} (SCSV3) and DNA-PK^{+/+} (SCH8-1) cells were treated with 20 Gy IR and collected at 30 min. Nuclear lysates were subjected to immunoprecipitation with anti-c-Abl. The immunoprecipitates were assayed for phosphorylation of GST-Crk(120-225). The results are expressed (mean $\pm$ s.e. of three determinations) as the IR-induced increase in c-Abl activity compared to that in unirradiated cells.

To assess further the functional significance of the interaction of c-Abl with DNA-PK and Ku, we investigated whether these proteins serve as substrates for c-Abl *in vitro*. Incubation of purified DNA-PK and Ku with c-Abl resulted in the phosphorylation of DNA-PK and not Ku (Fig. 3a and data not shown). Phosphoamino-acid analysis confirmed phosphorylation of DNA-PK on tyrosine (data not shown). Other studies have shown that autophosphorylation of DNA-PK inhibits its activity by inducing the dissociation of DNA-PK from DNA¹⁷. DNA-PK was bound to DNA-beads to assess the effects of c-Abl on the association of DNA-PK with DNA. The DNA-PK-containing beads were incubated in the presence of wortmannin to inhibit DNA-PK autophosphorylation and thereby autodissociation from DNA (Fig. 3b). The addition of heat-inactivated c-Abl had no detectable effect on release of DNA-PK from DNA (Fig. 3b). By contrast, incubation with kinase-active c-Abl resulted in release of tyrosine-phosphorylated DNA-PK from the beads (Fig. 3b). Whereas DNA-PK requires DNA for activity, these findings indicated that c-Abl downregulates DNA-PK by inhibiting the ability of DNA-PK to associate with DNA.

To determine whether DNA-PK is phosphorylated on tyrosine in cells, we assayed anti-DNA-PK immunoprecipitates for reactivity with anti-P-Tyr. The results demonstrate IR-dependent tyrosine phosphorylation of DNA-PK (Fig. 4a). These findings were confirmed when anti-P-Tyr immunoprecipitates were analysed by

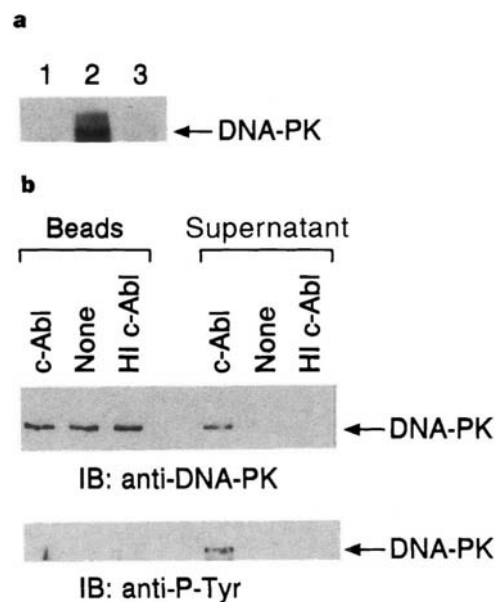


Figure 3 c-Abl phosphorylates and inactivates DNA-PK *in vitro*. **a**, Kinase-active c-Abl prepared from baculovirus²³ was incubated with purified DNA-PK/Ku complex. *In vitro* kinase reactions were analysed by SDS-PAGE and autoradiography (lane 1, DNA-PK/Ku; lane 2, c-Abl + DNA-PK/Ku; and lane 3, heat-inactivated c-Abl (HI c-Abl) + DNA-PK/Ku). **b**, Inactivation of DNA-PK by kinase-active c-Abl. Purified DNA-PK/Ku was incubated with DNA-beads. The beads were washed and suspended in kinase buffer. Kinase reactions containing beads, 20 μ M wortmannin, ATP and purified kinase-active or HI c-Abl were incubated for 15 min at 30°C. The supernatant fraction was obtained by sedimentation of the beads and washing in kinase buffer. The beads and supernatant fraction were boiled in SDS sample buffer. Proteins were separated by 5% SDS-PAGE and analysed by immunoblotting with anti-DNA-PK or anti-P-Tyr.

immunoblotting with anti-DNA-PK (Fig. 4b). Similar results were obtained in wild-type mouse embryo fibroblasts, but not in cells deficient in c-Abl (Fig. 4c). These findings provided support for IR-induced tyrosine phosphorylation of DNA-PK by a c-Abl-dependent mechanism.

The basis for activation of c-Abl by DNA damage¹⁻⁵ has been unknown. These findings demonstrate that the SH3 domain of c-Abl binds constitutively to DNA-PK and that the c-Abl/DNA-PK complex associates with Ku in response to DNA damage. Because DNA-PK is activated by DSBs^{8,15} and DNA-PK induces c-Abl activity, the results support a model in which DNA-PK contributes at least in part to c-Abl activation in the IR response. Moreover, in concert with a potential feedback mechanism, the results indicate that c-Abl downregulates DNA-PK activity by phosphorylating DNA-PK and thereby inhibiting association of DNA-PK with DNA. Cells defective in DNA-PK activity are hypersensitive to killing by IR as a result of ineffective DSB repair.⁹ By contrast,

cells deficient in c-Abl activity are resistant to IR-induced death¹⁸. Taken together with the functional interactions found between c-Abl and DNA-PK in these studies, these findings suggest that the balance between activation of these kinases could determine cell fate with repair of DSBs and survival or induction of cell death mechanisms. □

Methods

Cell culture. U-937 cells and mouse embryo fibroblasts (MEFs; *abl*^{+/+}, *abl*^{-/-})¹⁹ were grown as described previously¹. Mouse DNA-PK^{-/-} (SCSV3) and DNA-PK^{+/+} (SCH8-1) cells were grown as described previously²⁰. Irradiation was performed using a Gammacell 1000 (Atomic Energy of Canada) with a ¹³⁷Cs source emitting at a fixed dose of 0.21 Gy min⁻¹ as determined by dosimetry. Cells were also treated with 1 mM MMS (Sigma).

Immunoprecipitates and immunoblot analysis. Nuclear lysates were prepared as described in lysis buffer containing 1% Nonidet P-40 and 0.25% deoxycholate³. Immunoprecipitations were performed as described³ with anti-c-Abl (K-12; Santa Cruz), anti-DNA-PK (Ab 145, kindly provided by C. Anderson)²¹ or anti-Ku (GE2-9.5)²². Proteins were separated in 5% or 7% SDS-polyacrylamide gels and transblotted onto nitrocellulose. Immunoblot analysis with anti-DNA-PK (Ab 145), anti-c-Abl (Ab-3, Oncogene Science) or anti-P-Tyr (4G10; UBI) was as described³.

Fusion protein binding assays. Purified GST, GST-c-Abl (full length)²³ and GST-Abl SH3¹³ proteins (5 µg) were incubated with lysates from IR-treated cells for 2 h at 4°C. The adsorbates were analysed by immunoblotting with anti-DNA-PK (Ab 145).

c-Abl kinase assays. Nuclear lysates were subjected to immunoprecipitation with anti-c-Abl as described¹. The protein complexes were washed and resuspended in kinase buffer containing 2.5 µCi [γ -³²P]ATP and GST-Crk(120-225). Phosphorylation was analysed by SDS-PAGE and autoradiography.

In vitro kinase assays. Phosphorylation of c-Abl by DNA-PK: Purified DNA-PK/Ku complex (Lot 59166; Promega, Madison, WI) was incubated with heat-inactivated c-Abl with or without sonicated DNA in the presence of [γ -³²P]ATP and DNA-PK kinase buffer (25 mM HEPES, pH 7.5, 75 mM KCl, 10 mM MgCl₂, 1 mM DTT, 0.2 mM EGTA, 0.1 mM EDTA) for 15 min at 30°C. Reactions were terminated by adding 6 × SDS-PAGE sample buffer and analysed by SDS-PAGE and autoradiography. The phosphorylated c-Abl band was excised for phosphoamino-acid analysis.

Activation of c-Abl by DNA-PK: Active c-Abl was phosphorylated by purified DNA-PK/Ku as described above using unlabelled ATP. GST-Crk(120-225) fusion protein and [γ -³²P]ATP were then added for 15 min at 30°C. Proteins were separated in 10% SDS-polyacrylamide gels, transferred to nitrocellulose and analysed by autoradiography or by immunoblotting with anti-P-Tyr (4G10, UBI).

Phosphorylation of DNA-PK by c-Abl: Kinase-active c-Abl was incubated with purified DNA-PK/Ku (no sonicated DNA) and [γ -³²P]ATP in kinase buffer (20 mM HEPES, pH 7.5, 10 mM MgCl₂ and 10 mM MnCl₂) for 15 min at 30°C. Phosphorylated proteins were separated by 5% SDS-PAGE and analysed by autoradiography. The phosphorylated DNA-PK band was excised for phosphoamino-acid analysis.

Inactivation of DNA-PK by c-Abl: DNA-PK/Ku (20 µg, Promega) was incubated with double-stranded DNA-beads (25 µg; US Biochemicals) in kinase buffer (25 mM HEPES, pH 7.5, 75 mM KCl, 10 mM MgCl₂, 0.2 mM EGTA, 0.1 mM EDTA, 1 mM DTT) for 10 min at room temperature. The beads were then washed twice with kinase buffer. Kinase reactions containing beads, 100 µM ATP, 20 µM wortmannin (Sigma) and heat-inactivated or kinase-active c-Abl (2 µg) were incubated for 15 min at 30°C. The supernatant fraction was obtained by sedimentation of the beads. Following washing of the beads with kinase buffer, the beads and supernatant fraction were boiled with SDS sample buffer. Proteins were separated by 5% SDS-PAGE and analysed by immunoblotting with anti-DNA-PK or anti-P-Tyr.

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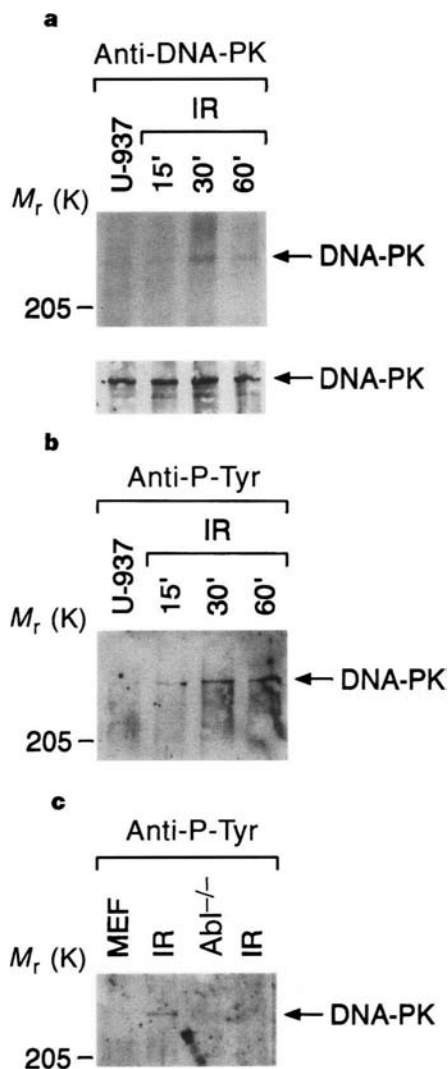


Figure 4 c-Abl-dependent tyrosine phosphorylation of DNA-PK in cells. **a**, U-937 cells were treated with 20 Gy IR and collected at the indicated times. Lysates were subjected to immunoprecipitation with anti-DNA-PK (Ab 145). The precipitates were analysed by immunoblotting with anti-P-Tyr (4G10, UBI) or by immunoblotting with anti-DNA-PK (Ab 145). **b**, Lysates were subjected to immunoprecipitation with anti-P-Tyr (polyclonal anti-P-Tyr Ab; UBI). The precipitates were analysed by immunoblotting with anti-DNA-PK (Ab 145). **c**, Wild-type and *Abl*^{-/-} MEFs were treated with 20 Gy IR and collected after 30 min. Lysates were immunoprecipitated with anti-P-Tyr and the precipitates were analysed by immunoblotting with anti-DNA-PK.

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***Drosophila* CBP is a co-activator of *cubitus interruptus* in *hedgehog* signalling**

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The transcription factor CBP, originally identified as a coactivator for CREB^{1,2}, enhances transcription mediated by many other transcription factors^{3–7}. Mutations in the human CBP gene are associated with Rubinstein–Taybi syndrome, a haploinsufficiency disorder characterized by abnormal pattern formation⁸, but the mechanism by which decreased CBP levels affect pattern forma-

tion is unclear. The *hedgehog* (*hh*) signalling pathway is an important determinant of pattern formation. *cubitus interruptus* (*ci*), a component in *hh* signalling, encodes a transcription factor homologous to the Gli family of proteins⁹ and is required for induction of the *hh*-dependent expression of *patched* (*ptc*), *decapentaplegic* (*dpp*) and *wingless* (*wg*)¹⁰. Haploinsufficiency for the *ci*-related transcription factor Gli3 causes phenotypic changes in mice (known as ‘extra-toes’¹¹) and humans (Greig’s cephalopolysyndactyly syndrome)¹² that have similarities to Rubinstein–Taybi syndrome. Here we show that *Drosophila* CBP (dCBP) functions as a coactivator of Ci, suggesting that the dCBP–Ci interaction may shed light on the contribution of CBP to pattern formation in mammals.

A dCBP complementary DNA was isolated by screening *Drosophila* libraries under low-stringency hybridization conditions with a *Caenorhabditis elegans* CBP probe. The deduced amino-acid sequence of dCBP predicts a protein of relative molecular mass 332,000 (332K) which includes all of the motifs found in the mammalian CBP/p300 homologues (data not shown). The dCBP gene was mapped to position 8F/9A on the X chromosome (data not shown).

A mutant of the dCBP gene, *nejire*^P (*nej*^P), was isolated as a P-element insertion through an enhancer trap screen. The P element was located 347 base pairs (bp) upstream of the second exon in the dCBP gene. Of 204 independent revertant lines obtained by remobilization of the P element, 53 lines were hemizygous lethal. Of these lethal lines, *nej*³, which is a deletion of 2–3 kilobases (kb) of genomic DNA near the 5′ end of the dCBP gene, shows the most severe phenotype and so we will confine our description mainly to this mutant. The heat-induced expression of dCBP from the *hs-dCBP* transgene was sufficient to rescue the *nej* zygotic lethal phenotype. The hemizygous *nej*³ embryo dies at stage 9 or 10 during embryogenesis, although some embryos survive to hatching. The most severe phenotype of the *nej*³ hemizygotes is the twisting of the embryo (embryonic length 0–70%) at germband elongation (the mechanism that generates the twisted embryo phenotype will be described elsewhere; H.A., D.-X.H. & S.I., manuscript in preparation).

To elucidate the role of dCBP in pattern formation, the expression patterns of several genes that are involved in establishing each parasegment were examined in the *nej*³ mutant. The expression of *wg* is strikingly reduced at the posterior margin of each parasegment (Fig. 1a, b). In addition, *engrailed* (*en*) expression, which is maintained by *wg* protein, is significantly lower than in wild type (Fig. 1c, d). These observations suggest that dCBP might contribute to the

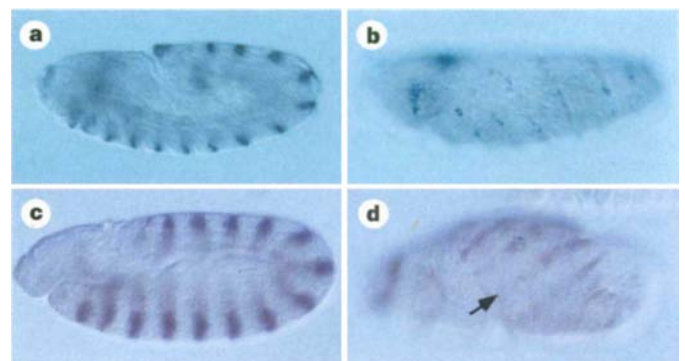


Figure 1 Decreased expression of *wg* and *en* in the *nej* mutant. Wild type (**a** and **c**) and zygotic homozygous *nej*³ (**b** and **d**) embryos were used for staining with anti-β-galactosidase. **a** and **b**, Pattern of *wg-lacZ* expression in stage-9 embryos. **c** and **d**, Pattern of *en-lacZ* expression in stage-10 embryos. An oblique stripe of cells, which express *en* at low level, is visible in the anterior portion of the mutant embryo (arrow). Anterior is to the left and dorsal is up.

function of some transcription factors involved in the *wg*-*en* signalling pathway.

To identify the factor(s) that might use dCBP as a coactivator to regulate *wg* transcription, we screened a *Drosophila* λEXlox expression library with a dCBP protein probe, and identified one clone encoding Ci. Interactions with Ci were also detected by screening a *Drosophila* embryo cDNA library in a yeast two-hybrid screen: of seventeen positive clones identified with a bait containing the dCBP CREB-binding domain, seven encoded Ci. Binding assays using a series of deletion mutants of Ci indicate that a region of Ci between amino acids 1,020 and 1,160 is required for phosphorylation-independent interaction with dCBP (Fig. 2a). This region has been defined as part of the Ci activation domain¹³. Co-transfection

of a *ci* expression vector into *Drosophila* Schneider cells together with the reporter plasmid pADCAT6GBS (containing six Gli-binding sites) increased the level of chloramphenicol acetyl transferase (CAT) activity in a dose-dependent manner (Fig. 2b). Addition of a dCBP expression plasmid augmented transactivation by Ci up to a maximum of 62-fold (Fig. 2c). In control experiments, dCBP did not affect CAT expression for the pADCAT6GBS reporter plasmid in the absence of Ci (Fig. 2c). In addition, CAT expression from a control reporter lacking the Gli-binding site was not affected by expression of Ci or dCBP (data not shown).

In the dominant gain-of-function mutant *ci^D*, the longitudinal vein 4 of the adult wing is shortened, some posterior row hairs are missing, and the posterior wing margin is flattened

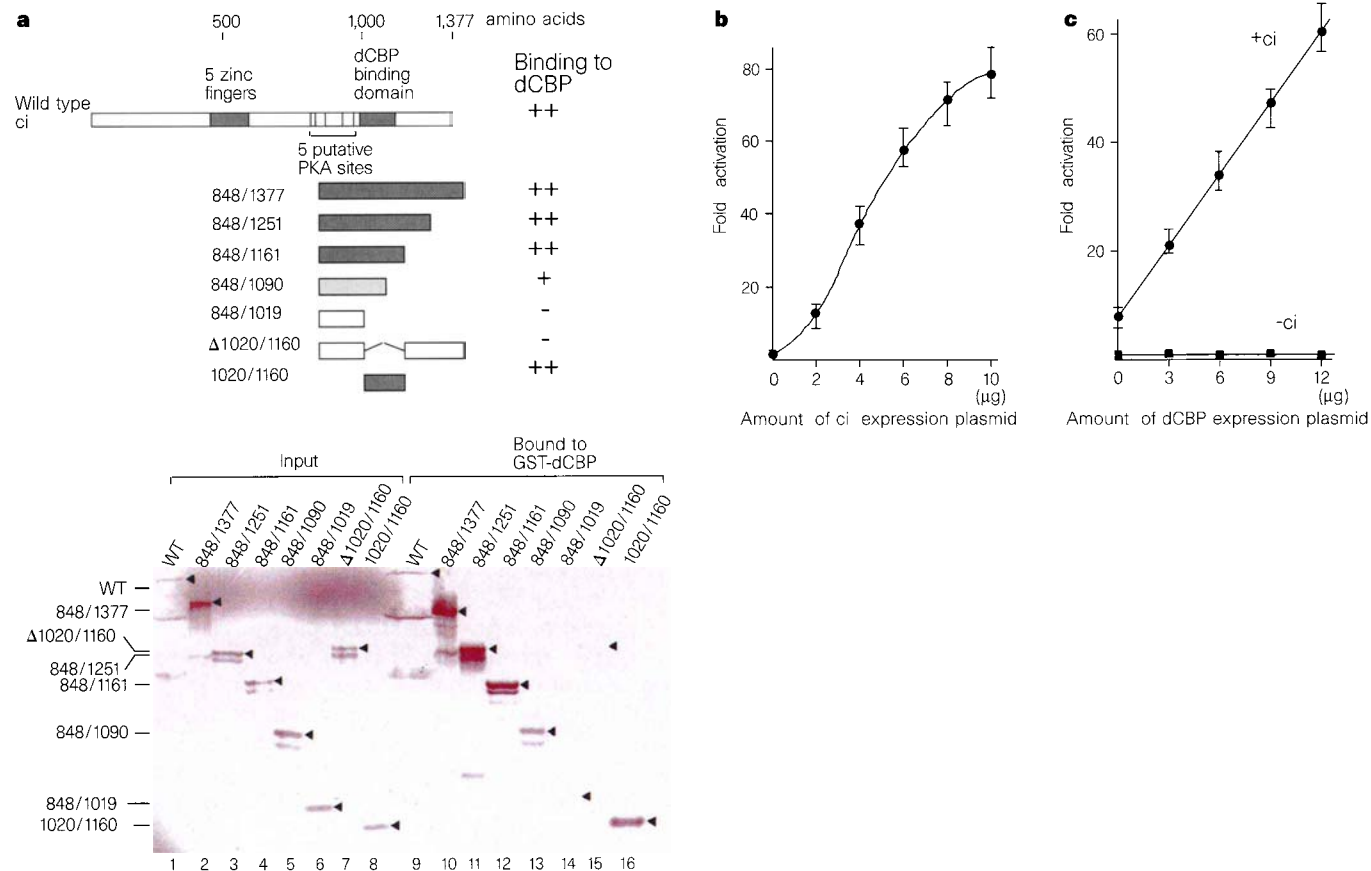


Figure 2 dCBP functions as a co-activator of Ci. **a**, Binding of Ci to dCBP. The structures of the Ci fragments analysed are shown. The relative binding activities are indicated to the right. The *in vitro* translated ³⁵S-Ci was mixed with the GST-dCBP379. Bound proteins were analysed by 10% SDS-PAGE followed by autoradiography. In input lanes, the amount of Ci was 10% of that used for the binding assays. **b**, Transcriptional activation by Ci. A mixture of increasing amounts of the

Ci expression plasmid pact5c-ci and the CAT reporter plasmid pADCAT6GBS was transfected into Schneider cells, and CAT was assayed. Data represent means ± s.e.m. **c**, Potentiation of the Ci-dependent transcriptional activation by dCBP. A mixture of increasing amounts of the dCBP expression plasmid pADCAT6GBS with pact5c-ci or control plasmid was transfected and CAT was assayed.

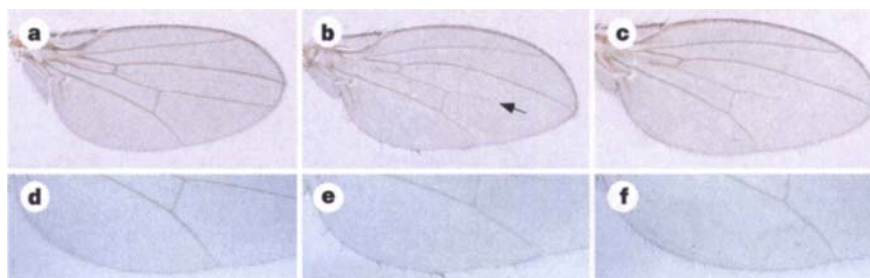


Figure 3 Genetic evidence for Ci-dCBP interaction. **a** and **d**, Morphology of wild-type wing. Higher magnification of a posterior margin is shown in **d**. **b** and **e**, The

wing of a *ci^D* heterozygote. Longitudinal vein 4 is shortened (arrow). **c** and **f**, The wing of a *nej³/+*; *ci^D/+*trans-heterozygote.

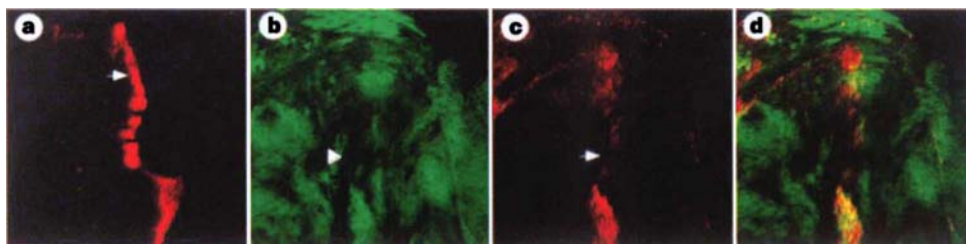


Figure 4 dCBP-deficient clones in the wing imaginal disc. *nej³* homozygous clones were visualized by the absence of Myc marker staining (green in **b**). Arrowhead indicates the *nej³* mutant clones that cross the A/P stripe of *ptc* expression. *ptc* expression was monitored in the same disc (**c**) and the control wild-type disc (**a**) by staining for *ptc-lacZ* expression. Arrows indicate the

corresponding regions in the wild-type and mutant disc where *ptc* expression is different (note that higher magnification was used for the mutant disc). Right panel (**d**) superimposes two single staining patterns. Anterior is to the left; ventral is up.

(Fig. 3a, b, d, e). This phenotype can be explained by the inappropriate expression of *ci* in the posterior compartment of the wing imaginal disc, where it is usually repressed by *en*^{14,15}. In the *nej³/+; ci^D/+* trans-heterozygotes, there was an increased number of posterior-row hairs and a normally shaped wing, although vein 4 was still shortened (Fig. 3c, f). Thus, a subset of the *ci^D* wing defects is suppressed by a haploinsufficiency of dCBP. Analysis of *ci*, *wg* and *ptc* expression indicates that the missing posterior row hairs are due to the inhibition of *wg* activity by the ectopic expression of *ptc*, and that the expression of *wg* is restored as a result of the decrease in *ptc* expression by a reduction of the Ci co-activator dCBP (data not shown). This result is consistent with the evidence that *ptc* over-expression inhibits the expression of *wg*¹⁶.

To prove that dCBP is required for the activation of *ci* target genes, *nej³* mosaic clones were produced in the wing imaginal disc and the expression of a *ci* target gene, *ptc*, was examined (Fig. 4). *ptc* is normally expressed at low levels throughout the anterior compartment except at the anterior/posterior (A/P) compartment border where there is a stripe of high expression (Fig. 4a). As shown in Fig. 4c, in the large *nej³* mutant clones across the A/P boundary, *ptc* expression is absent. These results support the idea that dCBP is a coactivator of Ci. Ci not only mediates the transduction of Hh, but also restricts Hh secretion to the posterior cells by inhibiting *hh* expression in anterior cells¹⁷. Consistent with this, Ci has both the activation and repressor domains in the C- and N-terminal region¹³. In *ci*-mutant clones generated in the anterior compartment of the wing, the resulting ectopic expression of Hh leads to wing duplication¹⁹. However, this type of duplication was not observed in dCBP-mutant clones, probably because dCBP is required for the activator function of Ci, but not for the repressor function.

Although protein kinase A (PKA) appears to participate in the signalling pathway that represses Hh target genes such as *dpp* (for review, see ref. 10), the mechanism underlying the antagonistic actions of PKA and *hh* is unknown. Our data indicate that dCBP binds to dCREB2, the *Drosophila* homologue of CREB, in a phosphorylation-dependent manner (data not shown), whereas the dCBP-Ci interaction is phosphorylation-independent. Our findings raise the possibility that a limiting amount of dCBP might be recruited to PKA-phosphorylated dCREB2, resulting in a decrease in Ci activity. A similar competition model involving mammalian CBP was proposed to explain the AP-1 inhibition of nuclear hormone receptors⁵.

Methods

Isolation of dCBP cDNA clones. A DNA fragment encoding a portion of the *C. elegans* CBP gene was used to screen a *Drosophila* genomic library (provided by J. Tamkun) at low stringency. The isolated clone was then used to probe a *Drosophila* cDNA library¹⁹. 5' RACE (rapid amplification of cloned ends) was performed to clone the 5' half of dCBP. To confirm the accuracy of the 5' RACE

sequences, an adult *Drosophila* head λ gt11 cDNA library (provided by P. Salvaterra) was screened using the RACE products and two cDNAs were isolated.

Immunohistochemistry. Fixation and staining of embryos and imaginal discs was essentially as described²⁰, except that a Vectastain ABC kit was used for signal detection.

Fly stocks. Isolation of *nej* mutants: several embryonic recessive lethal lines were generated by mobilization of the P-lacWelement into various locations on the X chromosome. *w; P[lw]*, *CyO/ScO* flies were mated to flies carrying a Δ 2-3 transposase-producing chromosome, and the resulting mutant X chromosomes were kept as balanced stocks with *FM7c*. To isolate *nej* deletion mutants, the P-lacWelement in the *nej* gene was remobilized. The enhancer trap lines used for examination of expression pattern in embryos and wing imaginal discs were *wg^{P0727}* (Bloomington Stock Center), *ryxho25 (en)¹⁸* and *ptc-lacZ²¹*. The *ci* mutant, *ci^D/M^{57g}* (ref. 14) was provided by J. Locke.

Generation of *nej* mutant clones in wing imaginal disc. Clones of *nej³* mutant cells were generated by *flp*-mediated mitotic recombination²². First instar larvae of the genotype *w; nej³, FRT18A/w; FRT18NM; ptc-lacZ/+; hsp70-flp/+* (to monitor *ptc-lacZ* expression) were heat-shocked at 37 °C for 90 min to induce mitotic recombination at the base of 1L. To induce *hsp70-N-myc* gene expression in the wing imaginal discs, wandering third instar larvae were heat-shocked at 37 °C for 90 min, allowed to recover at 25 °C for 60 min, and then dissected and stained with Myc and β -galactosidase antibodies. The frequency of the *nej³* clones was very low: one wing clone for 300-400 larvae dissected.

Screening for dCBP-interacting proteins and *in vitro* binding assays. A λ EXlox library, constructed from a 10-12-h *Drosophila* embryo mRNA (Novagen), was screened with a GST-dCBP379 protein probe containing the 379-amino-acid region (781-1,159) of dCBP. The yeast two-hybrid assay was carried as described²³. The 'bait' was a dCBP fragment (amino acids 825-1,043) fused to the LexA DNA-binding domain. The library was a 0-6-h *Drosophila* embryo cDNA fused to the GAL activation domain (pACT; provided by Greg Gollig). GST pull-down assays have been described⁶.

Co-transfection assays. Transfections in Schneider cells used the reporter plasmid pADCAT6GBS, constructed by inserting Gli-binding sites into the *Xba*I site of pAdhCAT²⁴. pA5c-ci and pA5c-dCBP were constructed by inserting the *ci* and dCBP cDNAs into pUchs-neoAct5c (ref. 25). The control plasmid pA5c0 lacks the cDNA insert. To examine Ci-induced transactivation, a mixture of increasing amounts of the Ci expression plasmid pA5c-ci, 5 μ g CAT reporter plasmid pADCAT6GBS, and 2 μ g internal control plasmid pA5c- β gal was transfected into Schneider cells. The total amount of DNA was adjusted to 17 μ g by adding control plasmid pA5c0. To examine the potentiation of the Ci-dependent transcriptional activation by dCBP, a mixture of increasing amounts of the dCBP expression plasmid pA5c-dCBP, 3 μ g pADCAT6GBS or the control reporter plasmid pADCAT lacking the Gli-binding site, 3 μ g pA5c-ci or pA5c0, and 2 μ g pA5c- β gal was transfected into Schneider cells. Total DNA was adjusted to 20 μ g by adding the control plasmid pA5c0. CAT activities were normalized to β -galactosidase activity.

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Correspondence and requests for materials should be addressed to S.I. (sishii@rtc.riken.go.jp) or R.H.G. (goodmanr@ohsu.edu). The GenBank accession number for the dCBP cDNA sequence is U88570.

correction

Airborne signalling by methyl salicylate in plant pathogen resistance

Vladimir Shulaev, Paul Silverman & Ilya Raskin

Nature **385**, 718–721 (1997)

The abbreviations used for methyl salicylate (MeSA) and salicylic acid (SA) led to confusion over nomenclature: MeSA is the methyl ester of salicylic acid and not a methylated derivative of salicylic acid (otherwise known as creosotic acid).

Evidence for life on Earth before 3,800 million years ago

S. J. Mojzsis, G. Arrhenius, K. D. McKeegan, T. M. Harrison, A. P. Nutman & C. R. L. Friend

Nature **384**, 55–59 (1996)

The model calculation presented in this Letter is corrected by Eiler *et al.* in Scientific Correspondence elsewhere in this issue¹.

- Eiler, J. M., Mojzsis, S. J. & Arrhenius, G. Carbon isotope evidence for early life. *Nature* **386**, 665 (1997).

erratum

Cloning of a disintegrin metalloproteinase that processes precursor tumour-necrosis factor- α

Marcia L. Moss, S.-L. Catherine Jin, Marcos E. Milla, D. Mark Bickett, William Burkhart, H. Luke Carter, Wen-Ji Chen, William C. Clay, John R. Didsbury, Daniel Hassler, Christine R. Hoffman, Thomas A. Kost, Millard H. Lambert, M. Anthony Leesnitzer, Philip McCauley, Gerard McGeehan, Justin Mitchell, Mary Moyer, Gregory Pahel, Warren Rocque, Laurie K. Overton, Frank Schoenen, Theresa Seaton, Jui-Lan Su, Janet Warner, Derril Willard & J. David Becherer

Nature **385**, 733–736 (1997)

The fourth author's name (D.M.B.) was accidentally omitted; he is in the Department of Molecular Biochemistry at Glaxo Wellcome Research and Development.

Also, reference 8 was not included in the list. This was by G. M. McGeehan *et al.*, "Regulation of tumour-necrosis factor- α processing by a metalloproteinase inhibitor", published in *Nature* **370**, 558–561 (1994).

Stochastic resonance in non-dynamical systems without response thresholds

Sergey M. Bezrukov & Igor Vodyanoy

Nature **385**, 319–321 (1997).

A typographical error was introduced into equation (8) which is correct as shown here

$$\text{SNR} = \frac{V_s^2 r(0)}{2 \Delta f_A} \exp\left(\frac{\sigma^2}{2}\right) \frac{2 + \frac{r(0)}{f_c} \sigma^2 \exp\left(\frac{\sigma^2}{2}\right) \sum_{n=1}^{\infty} \frac{\sigma^{2n-2}}{n! n}}{1}$$

Implements of immunology

Antibodies and assays are on review in this issue, including two human platelet antibodies, a benchtop antibody production system, several new peptide inhibitors and fluorogenic substrates for measuring apoptosis enzymes.

Biotrak human cytokine ELISA

From Amersham Life Science

The latest additions to this range include assays for IL-5, IL-12, IL-13, sIL-2R and TGF β 1

Each assay is said to offer time savings with protocols that can be completed in less than a stated five hours. Every kit provides sufficient reagents for 96 wells of a microtitre plate with, in general, only 50 μ l of sample needed whether from serum, plasma or cell culture supernatant. In addition to the new ELISAs, ten plate kits are now available for the Biotrak IL-1 β , IL-2, IL-6, IL-10, IFN γ and TNF α human cytokine assays. Other Biotrak cytokine assays include mouse cytokine ELISAs and high-sensitivity human cytokine ELISAs, which feature the unique Amdex high-sensitivity conjugate.

Reader Enquiry No. 100

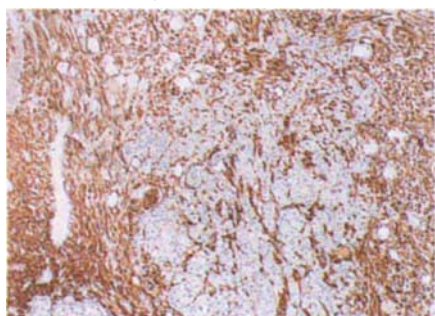
TRAcP antibody

From Zymed Laboratories

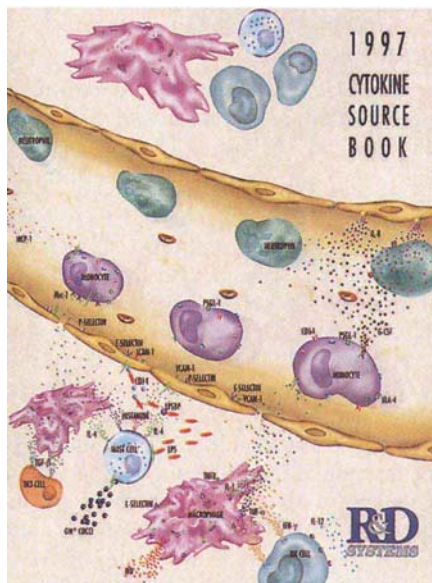
Detects hairy cells in paraffin-embedded tissue sections

Hairy-cell leukaemia (HCL) is a chronic, proliferative disease of the B-cell phenotype, which is diagnosed by the detection of leukaemia hairy cells. Enumeration of hairy cells also allows for the assessment of treatment response. Zymed Laboratories' new mouse monoclonal antibody to TRAcP (tartrate-resistant acid phosphatase) detects hairy cells of this leukaemia. The company states that this clone is the first commercial antibody to TRAcP that works well for paraffin embedded sections. This antibody was raised against the TRAcP antigen purified from the spleen of patients with hairy cell leukaemia. Positive staining has been observed in formalin- and B5-fixed material, including decalcified bone marrow biopsies. This antibody is available as a concentrate or as a ready-to-use reagent for use with Zymed's second-generation HistostainPlus detection systems.

Reader Enquiry No. 101



TRAcP antibody for HCL detection.



Also available from R&D Systems — the 1997 Cytokines Source Book.

Quantikine Tpo and ENA-78 ELISAs

From R&D Systems

Tpo (thrombopoietin) is believed to play a key role in the development of megakaryocytes

Tpo is secreted principally by hepatocytes and bone marrow stromal cells. This immunoassay is designed to measure the amount of natural and recombinant human Tpo in cell culture supernatant, serum and plasma samples, requiring an assay time of 3.5–4.5 h with a sample size of 200 μ l. It has a range of 62.5–2,000 pg ml⁻¹ and a sensitivity of <32 pg ml⁻¹. Another new assay from the company is the human ENA-78 ELISA. This immunoassay is a 4.5-h solid-phase ELISA, designed to measure human ENA-78 in cell culture supernatant, serum and plasma. According to the manufacturer, ENA-78, an alpha chemokine, is a neutrophil attractant and activator *in vitro*. With a sample size of 50 μ l and a sensitivity of 15 pg ml⁻¹, the ENA-78 ELISA should provide convenience, accuracy and reproducibility. Other available chemokine ELISAs include GRO α , IL-8, RANTES, MIP-1 α , MIP-1 β and MCP-1.

Reader Enquiry No. 102

PAC-1 platelet activation marker

From Becton Dickinson

Immunocytometry Systems

Two new human platelet antibody reagents: PAC-1 FITC and CD61 PerCP

Recognizing the fibrinogen binding site in the gpIIb/IIIa complex on activated plate-

lets, PAC-1 can be used in studies of platelet activation and aggregation, vascular injury, cardiovascular diseases and platelet disorders, as well as in monitoring cardiovascular therapies. CD61 is a pan platelet marker and is now available as a PerCP conjugate for flexibility in creating multi-antibody assays. A combination of PAC-1 FITC, CD62P PE and CD61 PerCP can be used to monitor gpIIb/IIIa activation and α degranulation simultaneously. In flow cytometry applications, these reagents allow platelet researchers to gain more information per sample, allowing multi-parameter capability whereby researchers can identify both the reactivity of platelets and resolve subtle stages of platelet activation. A direct, whole-blood assay is said to minimize the amount of sample manipulation, reducing artificial activation, as well as preserving the biological environment for more relevant results.

Reader Enquiry No. 103

Cytokine matched-pair antibodies

From Serotec

A new range of cytokine matched-pair antibodies for ELISA development

Serotec has launched a range of high-quality 'matched-pair' antibodies to enable researchers to develop ELISA assays for cytokine studies. According to the manufacturer, the range offers coating and detection antibodies for both human and mouse cytokine ELISA development. They are also said to be a cost-effective alternative to commercial ELISA kits. Serotec supplies sufficient coating antibody for up to 40 ELISA plates and detection antibodies, which are biotinylated to provide sensitivity and flexibility in assay design. Furthermore, the antibodies are available separately, with discounts available for antibodies purchased as a pair. Streptavidin conjugates are available linked to alkaline phosphatase or peroxidase. Recommended protocols are available upon request.

Reader Enquiry No. 104

New ELISAs for IL-7 and sICAM-2

From Bio Whittaker

Intended for research with adhesion molecules and/or cytokines produced by Bender Medsystems

IL-7 has many roles in the inflammatory process, including involvement in proliferation of pro-B cells and pre-B cells, proliferation of immature and mature thymocytes, differentiation of cytokine T cells, expression

of ICAM-1 on melanocytes, as well as other roles. The main applications for IL-7 are in the study of chronic lymphocyte leukaemia (CLL), cyclic thrombocytopaenia and Hodgkin's disease. The kit is suitable for 41 determinations in duplicate and requires a 100- μ l sample. ICAM-2 is involved with recirculation of LFA-1+ lymphocytes, initial T-cell adhesion with APC and target cells, and activation-induced cell death, following antigen re-exposure. The main areas of interest for ICAM-2 are for research into adhesion molecules, T-cell activation and/or activation-induced cell death (AICD). The kit is suitable for 41 determinations in duplicate and requires a 15- μ l sample.

Reader Enquiry No. 105

Automated systems

Cell-Pharm System 100

From Unisyn Technologies

Benchtop monoclonal antibody production system for research-scale production of monoclonal antibodies

Designed for screening cell lines in feasibility programmes or in the scale-up production of biomolecules, this system is capable of producing 50–250 mg of antibodies in a month, using hollow-fibre bioreactor technology. The system is said to eliminate concentration steps, and reduces labour and purification costs. This 0.18 m² self-contained instrument uses a sterile, disposable flow path, which includes a 0.14 m² bioreactor and an oxygenator. Its integral carbon dioxide flow adjustment and media temperature controller are designed to eliminate the need for an incubator. For those researchers looking for GMP validation, the Cell-Pharm System 100 is available with all the necessary tools and protocols to simplify validation process under good manufacturing practices. The instrument's critical flow path is also disposable and validatable. Bioreactor flow paths are available in 10,000, 30,000 and 70,000 molecular weight cut-offs for cell-expression applications. Additional automation is available when using the CellPharm Autoharvester with user-programmable recirculation or sampling. The Cell-Pharm product line includes a variety of different



Cell-Pharm System 100 from Unisyn.

models ranging from the small-scale production unit to the large-scale production unit capable of producing 1 g of monoclonal antibody per day.

Reader Enquiry No. 106

Dynatech immunoassay system

From Dynatech

Advanced integrated applications software developed further for total automation of ELISAs

Designed as an enhanced version of the company's time management software (TMS), this is an applications suite that controls all aspects of the Dynatech immunoassay system (DIAS) automated EIA processor. Over 32 enhanced functions are offered in the new version, TMS version 1.63. These include assay password protection together with an alert before executing a modified assay, multiple assays on a single plate, multiple wavelength reading and an enhanced fluids database and level sensing modules. The Windows software also aids access to the powerful automation features of DIAS through the GUI pull-down menus and context-sensitive help. TMS 1.63 also supports Spider, Dynatech's computer network patrol software, which has been designed to overcome the problems of manual plate transfer between sample processors and automated EIA plate processing equipment. Spider takes control of TMS' system manager, using the SPD generated work file to determine plate-specific assay protocols and maintain the chain of custody so important for good laboratory practice. TMS consists of a suite of five closely related applications. Assays are created in the assay manager, automatically scheduled through the system manager to avoid equipment conflicts and results displayed and analysed in data reduction.

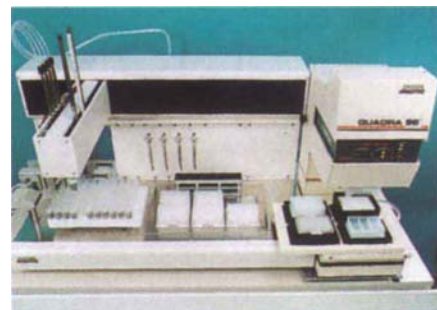
Reader Enquiry No. 107

Modular sample screening system

From Zinsser Analytic

Automates the rapid, high-throughput screening of samples

This system can perform weighing through to dissolving and plate production, as well as ELISA processing, all under full good-laboratory-practice compliant software control. Based on a robotic pipetting and plate handling, this system is modular and comprises four units that can be used individually or together in any combination to provide a flexible solution to individual requirements. The units include sample weighing and barcoding; solvent addition, dissolution and distribution to a 96-well master plate; production of daughter and/or dilution plates; and an ELISA processor. The software accepts data from up to 12 weighing stations, records the weight of each sample and the rack posi-



For high-throughput screening Zinsser Analytic offers a modular sample screening system.

tion of the sample tube. The information is matched to existing sample data, which can be downloaded from a central database. The data are used to calculate the solvent volume added to each sample at the second stage where dissolving is assisted by a vortex mixer or an ultrasonic bath. All pipetting is under software control and disposable pipette tips, which are used to avoid cross contamination. At each stage the sample position/barcode is checked to ensure that the position of a sample is known, right through from the initial weighing to the final ELISA processing and calculation of the test results.

Reader Enquiry No. 108

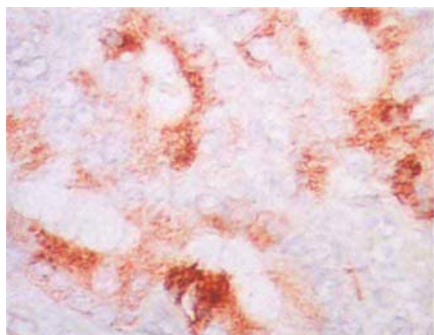
Cancer assays

Apolipoprotein-D prostate marker

From Signet Laboratories

A monoclonal antibody for the investigation of malignant and non-malignant human prostate tissues

This new monoclonal antibody, clone 8CD6, recognizes apolipoprotein-D (Apo-D), also known as gross cystic disease fluid protein-24 (GCDGP-24), a major constituent of high-density plasma lipoprotein and the major protein component of human breast cyst fluid. Apo-D, an androgen-regulated secreted protein, is widely expressed by prostatic adenocarcinomas, as well as a subset of breast, endometrial and ovarian neoplasms. Significant Apo-D immunoreactivity can be demonstrated in early stage prostate cancer and in advanced primary prostate tumours using the 8CD6 monoclonal antibody, while reactivity in the normal prostate gland and in benign prostatic hyperplasia (BPH) is negative. In normal tissues, Apo-D staining is restricted to the adrenal cortex, corpus luteum, peripheral nerves, pituitary, renal tubules and apocrine epithelium of axillary skin. In contrast to prostate-specific antigen (PSA) immunohistochemistry, which is said to discriminate unreliably between benign and malignant prostate cells, cellular Apo-D staining clearly distinguishes between non-malignant epithelial and malignant prostate cells in a large proportion of tumours and non-malignant



Signet's prostate marker — apolipoprotein-D.

prostate tissues, according to the manufacturer. This antibody may prove useful in classifying prostatic tumour subtypes, as an independent indicator of tumour progression, and as a reliable reagent for discrimination between malignant and non-malignant prostate cells. Signet's 8CD6 monoclonal reagent is supplied as a purified 1.0-ml immunoglobulin concentrate for use with Signet's detection systems, and may be applied successfully to routinely fixed, paraffin-embedded tissues, frozen sections or cytology specimens.

Reader Enquiry No. 109

Mouse HybriScreen IgG kit

From Biomed

A new hybridoma screening reagent set for the detection of mouse monoclonal antibodies of all IgG subclasses

This kit has been designed for use in mouse hybridoma supernatants or mouse ascites using enzyme immunoassay. Moreover, the manufacturer states that it will not detect monoclonal antibodies of IgA and IgM subclasses. The reagents in this kit detect primary antibodies of mouse isotypes IgG1, IgG2a, IgG2b and IgG3 origin in what is said to be a simple, antigen-mediated immunoperoxidase assay. This IgG assay is based on the antigen-mediated ELISA principle, using purified antigen that is adsorbed to the microtitre plate wells. The remaining sites for protein binding on the microtitre plate are saturated with blocking protein solution. This complex is then incubated with the antibody secreting hybridoma supernatants or diluted ascites. The mouse immunoglobulins bind to the antigen adsorbed to the wells and signal is detected by incubation with horseradish-peroxidase-labelled goat anti-mouse IgGs. This step is followed by incubation with ready-to-use liquid TMB (3,3',5,5'-tetramethylbenzidine) chromogen. A blue colour is developed in the TMB reaction with peroxidase within a few minutes and, if the reaction is stopped with a dilute acid solution, an intense yellow colour is formed. This system is stated to be sensitive down to 50 ng. A total of up to 10,000 tests (over 100 microtitre plates) can be performed with this reagent set.

Reader Enquiry No. 110

90K/Mac-2 BP ELISA

From Bender MedSystems

A new parameter for monitoring the outcome of cancer and virus infection

This is an ELISA for the quantitative detection of 90K/Mac-2, a widely expressed secreted 90K human serum glycoprotein, originally identified in the supernatant of human breast cancer cells. It is identical to Mac-2 BP, the ligand for the secreted lactose/galactose-specific S-lectin Mac-2, which is highly expressed by cells of the macrophage-monocyte lineage, as well as a variety of other cell types. Although the biological functions and possible roles of 90K/Mac-2 BP are largely unknown, some evidence indicates a role in the immune defence mechanism. Serum levels of 90K/Mac-2 BP have been determined in patients with various forms of neoplasia and in some viral infections. In breast and colorectal cancer and in non-Hodgkin's lymphoma, high levels of 90K/Mac-2 BP are correlated with a poor prognosis. In HIV infection, high serum concentrations of 90K/Mac-2 BP may serve as an indicator of faster progression to AIDS, independently of the numbers of CD4⁺ lymphocytes. According to the manufacturer, in a series of HCV-infected patients, a correlation was found between elevated serum levels of 90K/Mac-2 BP and failure to respond to treatment with α -interferon. The determination of 90K/Mac-2 BP in the serum can therefore be used as a valuable parameter for monitoring the outcome of cancer and viral infections. The kit is said to be suitable for the quantitative detection of human 90K/Mac-2 BP in culture supernatants, serum, plasma, and other body fluids. The kit contains all necessary reagents for 96 tests, making it easy and quick to use (a stated 185 min). The assay gives consistent reproducible results with a sensitivity of 1.4 ng ml^{-1} across a range of $12.5\text{--}200 \text{ ng ml}^{-1}$. The interference of circulating factors of the immune system is evaluated by spiking these proteins into 90K/Mac-2 BP-positive serum.

Reader Enquiry No. 111

Apoptosis research

Annexin V-FITC

From PharMingen.

Used to determine quantitatively the percentage of cells undergoing apoptosis

This assay relies on the property of cells to lose membrane asymmetry early in apoptosis. Apoptotic cells translocate the membrane phospholipid phosphatidylserine (PS) from the inner to the outer plasma membrane leaflet, exposing PS to the external environment. Annexin V, a Ca^{2+} -dependent phospholipid-binding protein, has a high affinity for PS and rapidly binds to

apoptotic cells with exposed PS. It identifies cells in an earlier stage of apoptosis than assays based on DNA fragmentation. The assay comes in 100- and 200-test sizes.

Reader Enquiry No. 112

Inhibitors and fluorogenic substrates

From Kamiya Biomedical

Several new peptide inhibitors and fluorogenic substrates for measuring the activity of enzymes involved in apoptosis

Among the enzymes that can be measured by these substrates are interleukin-1 β -converting-enzyme (ICE) inhibitor (VAD-FK), granzyme B inhibitor (AAD-CMK), and GPP-32/apopain inhibitor (DEVD-FK). These substrates are stated to be irreversible, highly specific and able to penetrate cell membranes. ICE substrate (YVAD-AFC), granzyme B substrate (AAD-AFC) and CPP-32 apopain substrate (DEVD-AFC) utilize a AFC coumarin derivative that makes the substrates both chromogenic and fluorogenic. A granzyme B fluorogenic enzyme overlay membrane (EOM) is also available for visualizing granzyme B isoenzyme activity patterns, following acrylamide or agarose gel electrophoresis.

Reader Enquiry No. 113

Monoclonal antibodies

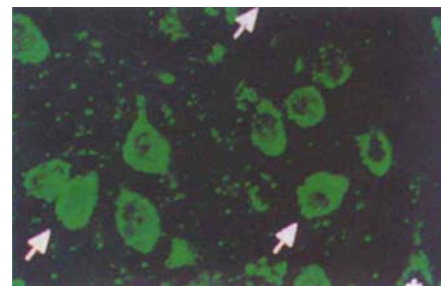
Glutamate receptor 2 antibody

From Chemicon

A mouse monoclonal antibody (Mab) to glutamate receptor 2 (GluR2)

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system. At least four classes of receptors mediate glutamate's action in the central nervous system. The major class of receptor, the AMPA receptor, is comprised of four distinct subunits (GluR1-4). The manufacturer has announced the availability of a new mouse monoclonal antibody to glutamate receptor 2 (GluR2), which is specific and shows no cross-reactivity to the other AMPA/Kainate GluR subunits. The Mab has been used successfully for western blot, immunocytochemistry and immunohistochemistry. Also available from the company are new monoclonal antibodies to GluR5,6,7 and NMDAR1 and polyclonals to mGluR1 α and mGluR5.

Reader Enquiry No. 114



Chemicon's monoclonal antibody to GluR2.

Polysciences' catalogue

From Polysciences

The catalogue offers some 200 new antibodies

Among these new additions are affinity purified antibodies that have been designed to meet the highest quality standards. Extensively cross-adsorbed to remove any unwanted reactivities, many can be useful for quantitative immunological measurements of clinically significant proteins and lipoproteins. The new antibodies are described in this new biochemical catalogue. Copies of this catalogue are available from the manufacturer, as are copies of Polysciences' *Particle, Polymers and Monomers* and *Microscopy/Histology* catalogues.

Reader Enquiry No. 115

c-Jun and p-JNK antibodies

From Autogen Bioclear

Two phospho-specific monoclonal antibodies produced by Santa Cruz Biotechnology: c-Jun and p-JNK

The c-Jun protein is said to be a major component of the transcription factor AP-1, originally shown to mediate TPA-induced expression of responsive genes through the TPA-response element that results in the expression of certain genes. JNK1 is activated by dual phosphorylation and functions to phosphorylate c-Jun. The phospho-specific monoclonal antibodies to these two proteins (c-Jun NH₂-terminal kinase) should enable the researcher to differentiate and analyse these steps within the signal-transduction cascade.

Reader Enquiry No. 116

Anti-Bcl-2 family of antibodies

From Trevigen

YtH series of antibodies for the detection of the Bcl-2 protein family members

The Bcl-2 family of proteins are important modulators of the apoptotic process and the relative ratio of these proteins has been hypothesized to determine cell fate. The company has monoclonal antibodies that are specific for known epitopes in Bcl-2 and Bcl-X_L that are specific for human, mouse and rat species. All of the antibodies are purified and have been qualified for use in western blot analysis and in immunoprecipitation. The antibodies are provided in liquid form and should be stored at 4 °C.

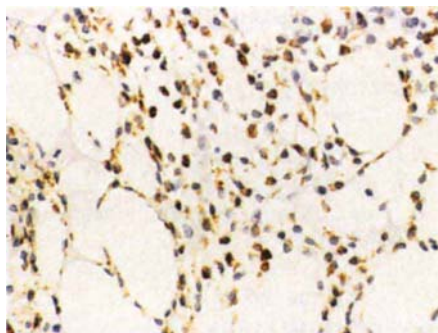
Reader Enquiry No. 117

Clone NGFR5 monoclonal antibody

From Dako

Monoclonal antibody against nerve growth factor receptor recognizes the 75K NGF receptor p75^{NGFR}

This p75 receptor protein is a member of the nerve growth factor/tumour necrosis factor



Antibody recognition of the nerve growth factor and tumour necrosis factor superfamily of receptors from Dako.

superfamily of receptors, many of which function as mediators of cell death, or apoptosis. *In vitro*, unbound p75^{NGFR} has been demonstrated to promote neural cell death, whereas, binding of p75^{NGFR} by NGF ligand or antibody has been shown to inhibit p75^{NGFR}-induced cell death. Immunohistochemical analysis with this antibody can be performed on routinely processed tissue sections, frozen sections or cell smears. Other apoptosis-related antibodies are also available from the company and include Bcl-2, p53, Lewis^y and NGFR.

Reader Enquiry No. 118

Deep-well plates

From Porvair Sciences

A microplate with the same 96-well footprint and format but different capacities

A family of microplates with differing well capacities that can all be used interchangeably on any liquid handling and automated sample processor is now offered by the company. The new plates have exactly the same height and use the standard format — once set there is no need to change the setting on the liquid processor.

Reader Enquiry No. 119

Inhibitors and fluorogenic substrates

From Kirkegaard & Perry Laboratories

Serum-adsorbed antibodies and cyanine conjugates

Xtra antibodies are affinity purified and adsorbed against bovine, chicken, goat, guinea pig, horse, human, mouse, rabbit, rat and sheep. They are said to show minimal cross-reactivity with these species and react strongly with the target antigen. The antibodies are available unlabelled or labelled with phosphatase, peroxidase, fluorescein, rhodamine, phycoerythrin and biotin. Affinity-purified antibodies to monkey immunoglobulins are also offered. New fluorescent conjugates include affinity-purified antibodies and streptavidin, avidin and biotin labelled with the cyanine dyes: Cy2, Cy3 and Cy5 and phycoerythrin. Due to the wide spec-

tral separation of their emissions, these conjugates are useful for multi-labelling experiments in flow cytometry and fluorescence microscopy.

Reader Enquiry No. 120

These notes are compiled by Brendan Horton from information provided by the manufacturers. For more details, fill in the reader service card bound inside the journal.

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